The Effects of Biochar and Anaerobic Digester Effluent on Soil Quality and Crop
Growth in Karnataka, India

THESIS

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Abstract

Both biochar and anaerobic digester effluent, which are byproducts of gasification and anaerobic digestion, respectively, have been proposed as a means of improving soil quality, plant productivity, and reducing C emissions. These soil amendments may be especially beneficial in rural regions of developing countries where biomass feedstock is locally available and energy poverty is pervasive. Thus, a field study was conducted in a remote village in Karnataka, India to test the ability biochar and anaerobic digester effluent to enhance soil quality and crop growth on a loamy sand soil. The two-year study included 4 treatments in the first year: control (Ct), biochar (B), effluent (E), and biochar+effluent (B+E) and two additional treatments in the second year: conventional fertilizer (C+F) and biochar+conventional fertilizer (B+F). The biochar was applied at a rate of 30Mg/ha and effluent at a rate of 56.25m³/ha. In the second year, 50kg/ha of NPK fertilizer was added to each conventional fertilizer plot. One year after application, soil ρ_b significantly decreased by 7.3% and f_T increased by 8.5% with biochar addition. Biochar also increased soil pH but had no impact on EC. The effluent did not have a significant effect on any soil properties tested, but after the first cropping season, biomass yield of radish (*Raphanus sativus L.*) from treatment E was approximately 11 times greater than Ct. Treatment B+E produced an even

greater yield, suggesting there was an interactive effect among soil amendments. However, in year 2, there were no significant differences in crop growth or soil N among treatments Ct, B, E, and B+E, nor were there differences in crop growth between C+F and B+F. The results indicate that anaerobic digester effluent has a positive effect on crop growth but that the biochar tested does not improve soil nutrient or water retention, suggesting additional research is required to determine the most effective forms of biochar for improving soil quality and increasing agricultural yields.

Dedication

I dedicate this thesis to Padmini Sekar, TR Krishna Kumar,
and Malathi Rajagopalan.

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Vita

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Fields of Study

Major Field: Environment and Natural Resources

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1 Introduction

1.1 Global Context

In the face of a rising world population and economic development, society is applying increasing pressure on the Earth's natural resources. Consequently, millions of individuals continue to face food insecurity and energy poverty. At the same time, fossil fuel-based development has led to anthropogenic climate change, which will have ramifications globally.

According to the United Nations Food and Agriculture Organization, the number of undernourished people around the world is over 1 billion, approximately 15% of world population, and food prices in 2011 have, again, increased to the peak levels reached in 2008 (Barrett 2010 and Ayre and Callway 2005). Energy poverty is also a ubiquitous challenge – 1.4 billion people do not have access to electricity, and 2.7 billion people rely on traditional biomass for cooking, which releases black carbon aerosols that cause respiratory illnesses and contribute to climate change (International Energy Agency 2010). It is also important to note that a major driver of rapidly rising food prices is increasing energy prices. Between March 2010 and March 2011, the price of crude oil increased 36%. Specifically, a 10% increase in crude oil price leads to a 2.7% increase in the World Bank Food Price Index due to a greater demand for biofuels, increased costs of inputs such as fertilizer and water, and higher transportation costs (The World Bank and Poverty Reduction & Equity Group 2011). Thus, food and energy availability for the world's rising population, are both serious challenges and inextricably linked.

The third critical issue being faced on a global-scale is climate change. The Intergovernmental Panel on Climate Change (IPCC) has determined with 90% confidence

that the radiative forcing, or the Sun's power per unit area, in the Earth's troposphere has increased between 0.6 and 2.4 W/m² since 1750. Increased radiative forcing can largely be attributed to a very large rise in atmospheric greenhouse gas (GHG) concentrations due to human activities such as the burning of fossil fuels or deforestation. These atmospheric changes have already increased the Earth's average temperature between 0.57°C and 0.95°C, and temperatures are projected to continue increasing at approximately 0.2°C per decade over the next century, leading to increased sea level rise, snow and ice melt, extreme weather events, and other climatic shifts that can be detrimental to many aspects of societal development, including food production (S. Solomon 2007). Agricultural practices, such as mineral fertilizer inputs, bore well irrigation, and biomass burning for energy can be both environmental hazards locally, causing air and water pollution and groundwater depletion, and significant contributors to climate change. Climate change can in turn exacerbate the severity of food insecurity. In order to combat these major environmental challenges, food insecurity, and energy poverty, it is necessary to adopt more environmentally sound agricultural inputs to improve soil fertility and crop yield.

1.2 Sustainable Development Programs in Rural India

Since the 1970s, the Indian Central government has instituted programs to promote the design and dissemination of several technologies aimed at improving energy access, soil fertility, public health, and the environment. While there have been some successes over the last three decades, 70% of Indians living in rural areas have still been underserved in these respects (Invernizzi and Foladori 2005). Three-quarters of these individuals continue to burn biomass in the form of firewood, cow dung, or crop residues for cooking, contributing to respiratory illnesses and black carbon emissions. Additionally, approximately 44% of rural

households depend on kerosene lamps for lighting instead of electricity (Ravindranath and Balachandra 2009). Two technologies that the Indian government commissioned to address these challenges were the biodigester (anaerobic digester) and the biomass gasifier, which are key elements of the following experiment.

Biodigesters utilize anaerobic bacteria to break down organic material such as cow dung or food waste into biogas and an effluent– a more thorough explanation will be presented in the following sections. The biogas produced from the digesters is largely composed of methane (CH_4) and can be used for cooking, lighting, or as an engine fuel, and the effluent can be used as an organic fertilizer. In 1982, the Indian government instituted the National Programme on Household Biogas, through which four million household digesters and 4000 community and institutional digesters have been installed throughout the country since 1985. It should be noted, that the only other country, which began a large-scale biogas deployment program in that time-period, China, now has 26 million biodigesters. Furthermore, a survey in the early 1990s found that only 66-87% of the digesters in India were still functioning (Bhattacharya and Jana 2009).

A second technology promoted by previous Indian government policies is the biomass gasifier. Gasifiers thermo-chemically convert woody biomass into a combustible gas, which can be used for internal combustion engines or electricity and charcoal production. A policy entitled The National Programme for Demonstration of Gasification Technology was put in place in 1987, and as of 2006, India had installed approximately 75MW of gasifier power. Initially, about 800 small wood gasifiers with a total capacity of 6.5MW were disseminated for irrigation water pumping, and after 1992 the gasifiers have been utilized largely for power generation. A total of approximately 1700 gasifiers have been installed to date

(Ravindranath and Balachandra 2009), and with regards to small-capacity gasifiers used for rural electrification, India has the largest program in the world (Bhattacharya and Jana 2009). Overall, while the successes of these programs have been measured, there has been significant penetration of biodigester and gasifier technology into India's rural areas. Thus, during the following experiment two of the byproducts of these technologies – biodigester effluent and charcoal – are utilized in a field trial in India to better understand the agricultural benefits of these soil amendments. Additionally, the thesis will holistically discuss the viability of these technologies to address the challenge of soil fertility in rural Indian villages.

1.3 Soil Quality

Soil quality refers to the soil's capacity to produce economic goods and services and regulate the environment (Lal 1994). Therefore, improving soil quality is commensurate with enhancing food security. Though soil quality in some areas of the world can naturally degrade over time due to erosion, unsustainable agricultural practices exacerbate erosion and other degradation processes. Soil quality can be divided into physical, chemical, and biological attributes, and increasing soil organic C (SOC) is an important mechanism through which all three aspects of soil quality can be enhanced (Lal 2009).

Biochar is a carbonaceous material that can provide the benefits of increased SOC in the long-term. Biochar is a product of pyrolysis, or thermal decomposition, of organic matter. Biochar is often a low density, porous material that has the potential to improve water and nutrient retention and provide a habitat for microorganisms in the soil, especially in degraded or sandy soils that do not inherently have those properties. The overall impact of biochar on soil quality is highly dependent on the biomass feedstock, the temperature of production, and several aspects of the soil being treated (Lehmann 2007).

Soil quality also has the potential to be significantly improved through the addition of anaerobic digester effluent, which is produced from the breakdown of organic matter by anaerobic bacteria (Kirchmann and Lundvall 1993). The effect of anaerobic digester effluent, also referred to as biodigester effluent or biogas slurry, on soil nutrient addition and soil quality is largely dependent on its feedstock and production process (Matsi et al. 2007; Graetz and Sweeney 1988). While this highly heterogeneous material is already popularly used as an organic fertilizer throughout the world, a dearth of information remains regarding the long-term effects of effluent addition on soil quality and SOC (Battacharya 2009; López-Mosquera et al. 2000; Lundvall et al. 2007).

1.4 Crop growth

Improving agricultural soil quality is a necessary intermediate step towards increasing crop yields. Thus, previous studies have also demonstrated the impacts of biochar and biodigester effluent on a variety of crops. Utilization of biochar and effluent can increase the soil concentration of plant available nutrients and water and enhance root growth environment (Yu et al. 2010; Kamman et al. 2011), ultimately improving crop productivity as a whole. However, there are limitations to the benefits that can be provided by biochar and effluent additions as well as limited knowledge of their wide array of impacts, which vary based on charcoal and effluent feedstock and soil and crop type (Lehmann and Joseph 2009; Dahiya and Vasudevan 1986). Few experiments have been performed examining the impact of applying biochar and biodigester effluent simultaneously on crop growth, indicating a strong need for further research on their potential to address global food challenges (Rodríguez and Preston 2009).

1.5 Energy and C sequestration

Both pyrolysis and anaerobic digestion are processes that facilitate the production of energy as well as an overall reduction in GHG emissions. Small-scale energy production in gasifiers and digesters address local energy needs. The application of biochar and digester effluent onto soils limit emissions directly as well as lessen the need for conventional fertilizer application and irrigation, which are highly energy-intensive practices. One-fifth of the global annual increase in radiative forcing is attributed to agriculture, and thus mitigating GHG emissions from the agricultural sector is necessary to minimize the effects of climate change (Cole et al. 1997).

1.5.1 Biochar

Previous studies suggest biochar can be a C sink and can be economically feasible as a soil amendment and source of energy. The production of biochar through pyrolysis stores approximately 50% of the C initially contained in the biomass. The remaining 50% of C is then available for biofuel production (Lehmann et al. 2006).

A life-cycle analysis by Gaunt and Lehmann (2008) found that adding biochar to soil is more effective in reducing C emissions than utilizing biomass for the sole purpose of bio-energy production. The analysis estimated that when 2024 MJ ha/yr and 2352 MJ/ha/yr were input into the production of wheat (*Triticum aestivum*) and corn (*Zea mays L.*), respectively, and their residues were used as a feedstock for pyrolysis optimized for biochar production, the char yields were calculated to be 534kg C/ha/yr and 599kg C/ha/yr. In both cases estimated energy yields were 6.9MJ/MJ (energy produced/energy input), and total avoided emissions due to the land application of biochars were 3768kg CO₂/ha/yr and 4227kg CO₂/ha/yr, respectively.

Biochar can be produced from various carbonaceous materials. Forest and crop residues, for example, would otherwise decompose in the soil releasing 80-90% of their C as CO₂ into the atmosphere. Therefore, in many cases pyrolyzing certain biomass for energy and biochar production acts as a C sink. Other “waste” materials such as sawmill residues, rice husks, and nutshells are also suitable for biochar production. Therefore, biochar can be made cheaply from these “waste” products and is also cost-effective in the sense that it will potentially increase crop production after being applied to the soil (Lehmann et al. 2006). It should be noted, however, that not all biochar production is definitively sustainable. Feedstocks retrieved from deforestation, for instance, could cause a net increase in GHG emissions. According to Gaunt and Lehmann (2008), even when pyrolysis is optimized for biochar production, 38% of the energy in the feedstock is converted to syngas – a mixture of gases, including but not limited to H₂, CO, H₂O, and CH₄ – which can in turn be utilized for energy generation (Ciferno and Marano 2002). While these opportunities for carbon sequestration and energy production are an important benefit provided by biochar, they are not the focus of this study. However, these value-added products and services will be discussed in the broader context of the practicality of utilizing biochar at the village-scale.

1.5.2 Biodigestion products

Anaerobic digestion is a process in which organic wastes are broken down to produce biogas, a mixture of CH₄, CO₂, and trace gases, and digested slurry. Converting organic wastes into these products reduces GHG emissions because the CH₄ and CO₂ are evolved in a digester where they can be captured and utilized for energy production (Clemens et al. 2006). Therefore, when organic matter is fully digested, C emissions from the

resulting digested slurries have been found to be less than those from undigested organic slurries applied to the soil (Amon et al. 2002). Utilizing anaerobic digestion to process cow dung is especially valuable in agricultural settings because the alternatives – storing cow dung in lagoons, burning cow dung paddies for energy, or cleaning indoor flooring with a cow dung-water mixture (an Indian cultural practice) – are not nearly as efficient or environmentally sustainable.

Organic residues such as grain husks, straw, and cattle dung are generally found in plenty on agricultural lands. However there are competing demands for these materials, anaerobic digestion has multiple advantages. Anaerobic digestion is one way to leverage the energy available in these organic wastes, which is a commodity that is lacking in many rural areas of developing countries. In 2007 China's biogas production was approximately 10.5 billion m³ from 26.5 million biogas plants, most of which were household digesters, not large-scale units (Chen et al. 2010). India has a technical potential of producing 36.8 billion m³ of biogas from its 600Tg of wet dung and other organic wastes annually. However, while there exist energy benefits – utilizing these wastes in the form of biogas produces more energy per unit of carbon emissions – often the biogas is used in inefficient cookstoves and furnaces or the biomass does not enter the biogas unit at all and is instead utilized in a traditional manner (Kishore et al. 2004). Thus, while biogas technology has great potential for energy production, there currently exist numerous limitations to its adoption at a village-scale.

1.6 Summary and Project Proposal

There is evidence that biochar and biodigester effluent have the potential to improve soil quality and crop growth while simultaneously addressing global challenges such as energy

availability and climate change. However, very limited information is available regarding the application of these soil amendments simultaneously and their interactions. Furthermore, additional research is necessary to understand the effect of biochar on soil properties, especially those of sandy soils (Novak et al. 2009). Thus, the following experiment was conducted over two years on a loamy sand soil in Karnataka, India. Both the biochar and biodigester effluent were produced on site from local resources. The purpose of this study is to understand the impacts of subabul (*Leucena leucocephala*) wood biochar and anaerobically digested cow dung effluent on soil quality and crop growth and to evaluate the use of these two soil amendments at a village-scale in India.

1.6.1 Site description

The experiment was located at 13°20'N and 76°39'E in Lakkihalli, Karnataka, India. Lakkihalli is a part of the Deccan Plateau where the major geological formations are composed granite gneiss. The average annual temperature in the region ranges from 20.3 to 27.6°C (Shiva Prasad 1998), and the annual rainfall is 453.5-717.7 mm, 55% of which falls during the monsoon season (Ramachandra and Kamakshi 2005). Soils in the area are Lithic Ustorthents (red non-gravelly loam). Lakkihalli is located in the hot moist semi-arid agroecological subregion with an annual growing period of between 150 and 180 days (Shiva Prasad 1998).

The local forest type is dry deciduous forest although there is very limited remaining natural vegetation in the area. The dominant forest species include oil-cake tree (*Albizia amara*), East Indian Satinwood (*Chlorozylon swietenia*), and red cutch (*Acacia chundra*). Principal crops in the area include finger millet (*Eleusine coracana*) and coconut (*cocos nucifera*) (Singh 1988).

1.6.2 Objectives

1. Examine the soil physical and chemical impacts of biochar and effluent additions,
2. Quantify the effects of biochar and effluent on crop growth, independently and concurrently, and
3. Evaluate the ability of biochar to retain nutrients in the long-term.

1.6.3 Hypothesis

1. Soil quality:
 - a. Biochar will reduce soil bulk density, increase water retention, and reduce soil compaction,
 - b. Biochar will increase pH, EC, and soil N, and
 - c. Effluent will increase pH, EC, and soil N.
2. Plant growth:
 - a. Biochar will increase all plant growth parameters and
 - b. Effluent will increase all plant growth parameters
3. Long-term effects: Biochar will allow for increased nutrient retention in soil

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2 Biochar and anaerobic digester effluent impact on soil properties

Abstract

Previous studies have suggested that biochar and anaerobic digester effluent have the potential to improve the soil quality in the long-term while simultaneously addressing pressing environmental challenges. A field experiment was conducted in Karnataka, India to assess the effects of these amendments on soil physical and chemical properties over a 15-month period. In year 2, biochar (B), which was added at a rate of 30Mg/ha the previous year, significantly decreased ρ_b by 7.3% compared to the control (Ct) and increased f_T by 8.5%. This effect was diminished when biochar was added along with effluent (B+E) (56.25m³/ha). Neither biochar nor effluent (E) increased w. Biochar increased soil pH from 7.21 (Ct) to 7.57 (B) and 7.50 (B+E), and the effects of both amendments on EC were inconsistent. Soil N was also unchanged after the application in all treatments. One year after application of amendments, soil physical properties and pH were slightly influenced by biochar addition, but there is no evidence that soil nutrients, such as cations or N, were retained with biochar addition.

2.1 Introduction

2.1.1 Biochar production and characterization

Biochar is a carbon rich material formed through the thermal decomposition of organic matter. Organic matter is heated in an oxygen-limited environment to produce charcoal as well as some liquid and gas byproducts. The exact chemical and biological effects of thermal decomposition are still quite nebulous. However, some of the impacts of biochar on carbon storage and crop productivity are encouraging and prompt further research (Lehmann and Joseph 2009).

As described by Raveendran et al. (1995), the mineral contents of biochar are greatly affected by the biochar feedstock. For example, biochars formed from straws and husks often have high silica (Si) content, and those formed from coconut coir have high potassium (K) and sodium (Na) contents. Rice (*Oryza sativa*) husk biochar has an inordinately high zinc (Zn) content, for example, and if the biochar is able to make the Zn more bioavailable, the use of biochar from rice husk could be especially beneficial in many developing countries where there is wide spread Zn deficiency (Arthur et al. 2003). However, the conditions under which biochar is able to make various nutrients bioavailable are yet to be determined.

Furthermore, physical characteristics of biochar are also influenced by the methods of biochar production. Surface area, for example, which affects biochar's adsorption properties and its ability to influence soil physical properties is greatly affected by and the temperature at which thermal decomposition occurs and the activation time of biochar. Activation, which is not necessary for the production of biochar, refers to a process of oxidation using gases such as steam or CO₂, which creates pores in the biochar (Lehmann and Joseph 2009).

2.1.2 Biochar impact on soil physical properties

Soil structure affects the ability of soil to retain water and nutrients, and it provides a growing environment for microorganisms and plants (Lal 1991). As a recalcitrant material with high surface area and porosity, biochar can have a major impact on soil physical properties. Many studies have found that biochar treated soils have a lower bulk density (ρ_b) and greater soil water holding capacity and aggregate stability (Zhang et al. 2011; Case et al. 2012; Piccolo et al. 1996; Glaser et al. 2002).

Soil ρ_b , or the weight of soil per unit volume, is an important measure of soil quality because it affects root growth, soil aeration, soil-water regime, runoff and erosion (Lal et al. 2001). Zhang et al. (2011) reported that adding 40Mg/ha of biochar to a calcareous Aquic Fluvent soil caused a decrease in ρ_b from 1.37g/cm³ to 1.09cm³ without the presence of N fertilizer and from 1.36g/cm³ to 1.13g/cm³ with N fertilization. Case et al. (2012) also found a decrease in the ρ_b of a sandy loam soil from 0.95g/cm³ to 0.84g/cm³. These results are expected because most biochar have very low bulk densities, often below 1.0g/cm³ (Lehmann and Joseph 2009).

Biochar often also has a high surface area and porosity, allowing for it to retain large amounts of water. The production of biochar involves heat treatment that increases porosity by dispelling low molecular weight volatiles between the crystalline carbon structures of biochar (Lehmann and Joseph 2009). Thus, as Kishimoto and Sugiura (1985) calculated, charcoal surface areas are between 200 and 400m²/g. Generally, an increase of porosity and surface area also lead to an increase in water retention (Troeh and Thompson 2005). A study performed on the effect of coal-derived humic substances, which are carbonaceous, largely recalcitrant materials like biochar, were found to significantly increase soil water holding capacity (WHC) and aggregate stability when added at rates of less than 1.0g/kg (Piccolo et al. 1996). Another similar experiment found that a 1.5Mg/ha addition of coal-derived humic substances was able to increase soil macro-aggregate stability up to 130% (Glaser et al. 2002).

Several experiments have since confirmed that biochar also has the ability to improve soil WHC. According to Kammann et al. (2011), the WHC of a sandy soil increased by 23.9% with the addition of 100Mg/ha of biochar and 36.1% with 200Mg/ha. Though it

should be noted that these biochar application rates are exceptionally high. Like results were also achieved with the addition of just 5Mg/ha on a loamy sand soil – WHC increased from approximately 5% to greater than 20%. Importantly, increased rates did not further improve WHC possibly because of hydrophobic characteristics displayed by the biochar, which are dictated by feedstock (Dugan et al. 2010). Glaser et al. (2002) also observed that biochar only improved moisture retention in sandy soils and caused a decline in WHC in clayey soil. Thus, an increase in surface area is only beneficial to soil WHC when it attracts polar water species and may not respond to all soil types consistently.

Aggregate stability is defined as the measure of cohesion between primary soil particles and their ability to withstand disruptive forces such as rainfall or wind (Kemper and Rosenau 1986). The coal-derived humic substances described above were also found to increase aggregate stability by 40-120%, as determined through wet sieving and the Kemper & Rosenau (1986) equation (Piccolo et al. 1996). Glaser et al. (2002) explained that the effect on aggregate stability can largely be explained by the formation of organo-mineral complexes between soil particles and humic acid fragments. Specifically, the fragments' hydrophobicity limit the ability for water to enter the aggregate pores.

2.1.3 Biochar and soil chemical properties

To demonstrate differences among biochars on soil chemical characteristics, Chan et al. (2008) compared the effects of two poultry litter biochars, one produced at 450°C and one at 550°C preceding activation, on the chemical properties of an Alfisol. The biochar produced at 450°C was more effective at increasing soil C and N even without the addition of N fertilizer, suggesting that the N contributed was largely from the biochar

itself. Other studies conducted on sandy and tropical soils with limited organic C content also reported increases in SOC compared to the control, however, it is not a fully consistent result of biochar addition (Zhang et al. 2011; Sukartono et al. 2011; Kimetu et al. 2008).

The effect of biochar on soil N concentration is also inconsistent. For instance, Chan et al. (2007) found no effect on soil N concentration when greenwaste biochar was added at 0, 10, 50, or 100Mg/ha onto a low C Alfisol. One explanation could be the small difference in N concentration between the charcoal and the soil. However, it is important to note that in this study there is no data available regarding the N content of the soils amended with biochar in conjunction with N fertilizer, and thus the Chan et al. (2007) experiment does not assess the ability of biochar to retain nutrients. Similarly, the addition of coconut shell biochar onto soil with a sandy loam texture also yielded no increase in soil N content, but cattle dung biochar added to the same soil did cause a slight but significant increase in soil N concentration, but the difference was no longer present after the second maize harvest (Sukartono et al. 2011).

Furthermore, a study measuring the impacts of a wood biochar on a savannah Oxisol did not report an increase in soil C or N concentrations after the application of biochar during any of the three years when the experiment was conducted. In fact, in the first year, total C and N concentrations were greater in the control plots than in the biochar amended plots (Major et al. 2010).

The effect of biochar on soil pH is typically more consistent though. While the pH of biochar can vary considerably based on feedstock and incubation period, most published studies have utilized alkaline biochar that demonstrated an increase in soil pH (Lehmann

and Joseph 2009). A recent meta-analysis of biochar studies found a statistically significant increase in crop productivity with the addition of biochar to acidic soils, suggesting the commonly alkaline nature of the material and its positive impact on soil quality (Jeffery et al. 2011). Nevertheless, Zhang et al. (2011) found that adding 20 and 40Mg/ha of wheat straw biochar to an alkaline soil had no effect on pH. The biochar had a pH of 10.4, but did not further increase the soil pH at either rate of addition.

Several other soil chemical properties are affected by biochar addition as well. Soil CEC, for example, is often increased with biochar amendment. Thus, an increase in cations such as K^+ , Ca^{2+} , and Mg^{2+} follow. Uzoma et al. (2011) found that the application at all rates (10, 15, 20Mg/ha) of a cow dung-based biochar on a dry land sandy soil significantly increased soil K^+ , Ca^{2+} , and Mg^{2+} concentrations. In addition, CEC increased with increasing application rate. It is important to note that the biochar in this study had more than 2 times the concentrations of all three cations (.14 cmol(+)/kg of K^+ , 2.12 cmol(+)/kg of Ca^{2+} , and 1.40 cmol(+)/kg of Mg^{2+}) than that of the soil (.06 cmol(+)/kg of K^+ , .34 cmol(+)/kg of Ca^{2+} , and .17 cmol(+)/kg of Mg^{2+}), and the CEC of biochar was 4.84 cmol(+)/kg, almost 8 times that of the soil. Though increased CEC is a common result of biochar addition, there is no definitive pattern on the effects of biochar (of any feedstock and temperature) on CEC and K^+ , Ca^{2+} , and Mg^{2+} contents of soil.

Though there is limited information on the effects of biochar on soil electrical conductivity (EC), EC is an important soil quality indicator that has the potential to be impacted by biochar addition. Soil EC is a measure of salinity. Exposure to highly saline soils can be deleterious to plant growth. Biochar EC is greatly influenced by production temperature and feedstock. An increase in production temperature from 350°C to 800°C

leads to an increase in Ponderosa Pine biochar EC from approximately 150 μ S/m to almost 20,000 μ S/m, likely due to the filling of the increased pore space (a result of increased HTT) with ash residue that contains a large quantity of salts. The same study found that Ponderosa Pine biochar had a significantly great EC than Douglas fir (*Pseudotsuga Menziessi*) biochar at both temperatures (Gundale and DeLuca 2006).

2.1.4 Biodigester effluent production and characterization

There are several processes and microorganisms involved in anaerobic digestion. Some processes include hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Cherosky 2012). The bacterial community necessary for each process is different. Ultimately, the products of these reactions – biogas and effluent – are characterized by the feedstocks into the digester, the type of digester, and the temperature at which it operates (Zhang et al. 2011).

Organic byproducts, which contain carbohydrates, fats, and proteins, are first broken down into simpler forms through hydrolysis. Following hydrolysis, acid-producing bacteria further break down the compounds into fatty acids such as acetic acid.

Methanogenic bacteria combine the products of those reactions: fatty acids, H₂, CO₂, and H₂O to form CH₄ and an effluent (Hamilton 2009). Digestion can occur at a range of temperatures between 20°C and 70°C. Thermophilic bacteria, a less common form, function between 45°-70°C, and thermophilic digestion occurs 2-4 times faster than digestion at a lower temperature. Mesophilic bacteria, however, have a more robust capacity to break down a diverse range of feedstocks, or substrates. Mesophilic digestion occurs between 20°C and 45°C. In order for the digestion process to be completed successfully, the multiple communities of anaerobic bacteria must be able to grow in a

balanced fashion and function synchronously. After the removal of CH₄ and CO₂ as well as some H₂S and NH₃, the remaining constituents of the digested feedstock makes up the effluent (Schanbacher 2009), which can have varying effects on soil fertility.

2.1.5 Effect of effluent on soil chemical properties

The soil quality improvements attributed to anaerobic digester effluent addition are largely related to nutrient applied through the effluent. Yu et al. (2010) found that both biodigested effluent as well as concentrated digested effluent (passed through a sieve) increased total available N in a sandy loam soil compared to the control. Möller et al. (2008) similarly reported that soil mineral N increased more with the addition of digested cow dung slurry than undigested manure slurry. However, other studies did not corroborate these results. Another study noted that soil mineral N significantly increased compared to the control only at the higher rate of effluent application, an addition in which N content was 150 mg N/dm² (Gunnarsson et al. 2010). Lundvall et al. (2007) found that a chicken manure-based effluent did not increase soil nitrate concentration to a greater extent than undigested manure. Importantly, this study specifically tested nitrate whereas other studies tested for total N. However, Hart (1963) suggested that N is concentrated during the digestion process and converted from an organic to form of ammonium by the bacteria in the digester. Therefore, measurement of total N would account for the ammonium-N, while measurement of nitrate-N would not.

Some studies have also suggested that anaerobic digester effluent addition increases soil OM concentration. When cow manure effluent or codigested effluent (cow dung+maize silage) was added to sand and loam soils, they produced a 10% increase in soil OM in both soils (Bachmann et al. 2011). Furthermore, Yu et al. (2010) found that an addition of

cattle dung slurry increased the percent soil OM to levels greater than both the control as well as the conventional fertilizer amended plots.

Finally, biodigester effluent can have a significant long-term impact on soil quality through its contribution to soil salinity. Yu et al. (2010) found that soil fertility was, in fact, positively correlated with EC. For instance, the addition of concentrated biogas slurry increased the EC from 89.4s/cm to 157s/cm, and there was a corresponding significant increase in available N, P, and K. However, crop yield was not positively influenced by soil EC, which was potentially linked to increased concentration of secondary nutrients contributed by the concentrated biogas slurry.

Biodigester effluent is a highly heterogeneous substance. Its impacts on soil quality can be numerous and varied depending on feedstock-type. However, if produced from agricultural wastes, the effluent will likely act as an organic nutrient fertilizer that improves soil quality in the short-term.

2.2 Materials and methods

2.2.1 Experimental design

A complete randomized block design was utilized, including 7 replications of 4 treatments: control (C), biochar (B), effluent (E), biochar+effluent (B+E). Each plot was 2x2m, and the buffer zone between plots was 1m in width. The plots were formed into beds in order to ensure that the soil hardpan did not interfere with plant growth.

In January 2011, 30Mg/ha biochar was added to each B and B+E plot, and 56.25m³/ha of effluent were added to each E and B+E plot. Additionally, for those plots that required both biochar and effluent the biochar was initially soaked in the effluent for 72 hours before addition. All plots, including control, were hand-tilled after the addition of soil amendments. The site had not been utilized for crop growth for the several years prior to the experiment. The first radish (*Raphanus sativus L.*) cropping season was between January and March 2011. The radish was of the *Pusa Chetki* variety. Furthermore, in January 2012, fertilizer and cow manure were added to 3 of the 7 replicates, resulting in an additional 4 treatments: NPK (C+F), biochar+NPK (B+F), effluent+cow dung, biochar+cow dung. The soil and plant effects of the cow dung treatments have been omitted. After adding these amendments to the soil, the second radish crop was planted. The radish variety in the second season, *Pusa Chetki Long*, was slightly different than the first but was produced by the same institute. The field was left fallow between the two seasons.

2.2.2 Biochar production

The biochar was produced in a traditional method from *subabul* wood. Though a gasifier was available on location, it was not functional. Thus, the highest treatment temperature (HTT) of this biochar is unknown.

2.2.3 Biodigester effluent production

The effluent was produced in a biogas digester located on-site. Cow dung, which was also available on-site, was used as the feedstock for the digestion process. The effluent was produced in a fixed dome digester.

2.2.4 Particle size analysis

Soil texture was determined using the pipette method (Gee and Or 2002). Because the texture was expected to be sandy, 20.2g of soil sieved to 2mm was used for the analysis. H_2O_2 was added to the soil water solution to remove any organic matter, and the samples were kept at 90°C to facilitate the reaction. Subsequently, the samples were shaken in a horizontal shaker with distilled water and Na-hexaphosphate in order to disperse the flocculating agents in the soil. After 24 hours, the particles were separated out of the sample with a 53µm sieve. Following the removal of sand, the soil water solution was left undisturbed in a 1L graduated cylinder. The solution was hand-stirred for 60s and allowed to settle for 4m and 35s before pipetting out the coarse silt fraction of the sample. The fine silt and clay were removed in a similar fashion 4 h after stirring.

2.2.5 Bulk density and field capacity

Soil cores were taken at the 0-10cm depth from 3 randomly chosen locations on each plot 24 hours after hand-watering the plots to saturation. The samples were weighed

immediately after collection. The samples were then sun-dried (at approximately 50°C) to a constant weight to determine soil dry mass and gravimetric water content. Additionally, soil porosity (f_i) was determined based on the calculated bulk density and an assumed soil particle density of 2.65g/cm³.

2.2.6 Soil pH and EC

Soil pH and EC measurements were taken 1 year after treatments were applied immediately prior to the second cropping season and were determined according to the procedure outlined by Sparks (1996). Soil:water solutions were prepared at a 1:1 ratio and shaken for 30s in the Fisher Scientific Vortex. The samples were left undisturbed for 10 minutes before measurements were taken. A Thermo Scientific Orion 4 star instrument was utilized for pH and EC measurements.

2.2.7 Total N

The soil residual N was evaluated one year after treatments were added in 2011. Soil samples were sieved to 2mm and spun on a SampleTek Vial Rotator to crush and homogenize the sample. Approximately, .250g of the samples were separated for analysis by combustion at 900°C (Nelson et al. 1996) using a CN Elemental Analyzer (Vario Max, Elementar Analysensysteme, Hanau, Germany).

2.2.8 Statistical analysis

An analysis of variance was conducted among treatments utilizing the PROC GLM procedure in Statistical Analysis Software 9.2 (SAS Institute, 2008). Least Significant Difference (LSD) values were also calculated using the same procedure. The results in sections 2.3.1, 2.3.3, and 2.3.4 are data based on 4 replicates of each 1st year treatment (Ct, B, E, and B+E; referred to as t1 treatments) and 3 replicates of the conventional

fertilizer treatment, added in the 2nd year (C+F and B+F; referred to as t2 treatments).

Section 2.3.2 refers to data from 7 replicates of t1 treatments.

The graphs were produced using STATA 12.1 (STATA Corp., 2011).

2.3 Results and discussion

2.3.1 Particle size analysis

The soil texture class of the Lakkihalli, Karnataka soil was determined to be loamy sand based on the PSA (Table 2.1). This texture determination is not in accordance, however, with the soil map identification of soils as red non-gravelly loam (also termed fine sandy loam) (Siva Prasad 1998). In either case, the soil has a significant percentage of sand – the boundary between sandy loam and loamy sand occurs at 70% sand. The soil map does refer to this area as well drained, which is logically in agreement with the result of the texture analysis.

Treatment	%Sand	%Silt	%Clay
C	82.2 ^a (0.6)	13.3 ^a (0.4)	4.5 ^a (0.9)
B	80.3 ^b (0.8)	12.8 ^a (1.6)	6.9 ^a (1.6)
E	80.4 ^b (0.4)	12.2 ^a (1.2)	7.4 ^a (0.9)
B+E	82.89 ^a (0.2)	12.6 ^a (0.5)	4.5 ^a (0.5)
LSD	1.7	3.2	3.3
p-value	0.01	0.91	0.15

Table 2.1 Soil texture; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n = 4 (aggregated from multiple locations on each plot)

The hydrologic properties of sandy loam and loamy sand soils are similar. Porosities, water retention at -33 and -1500kPa , and saturated hydraulic conductivity (K_s) are shown

in Table 2.2. These properties indicate that the soils in this experiment have a very limited ability to retain water and nutrients. Therefore, adding biochar to the soil could be beneficial to improving this soil's ability to make water and nutrients more plant available. This will be discussed further in the next section.

Texture class	Total porosity (cm ³ /cm ³)	Effective Porosity (cm ³ /cm ³)	Water retention at -33 kPa (cm ³ /cm ³)	Water retention at -1500kPa (cm ³ /cm ³)	Saturated Hydraulic Conductivity (cm/h)
Loamy sand	0.437	0.401	0.125	0.055	6.11
Sandy loam	0.453	0.412	0.207	0.095	2.59

Table 2.2 Soil hydrologic Properties; source: (Rawls et al. 1982)

The PSA results suggest that soil texture was significantly different among treatments. Relatively few studies have discussed the effect of biochar on soil texture, however. A study by Oguntunde et al. (2004) on biochar application to a loamy sand soil also found an increase in %sand from 78.4% to 83.8%. These results are consistent with the change from treatment E to B+E but not C to B. Because there is both an inconsistency with regards to the effects of biochar and effluent on soil texture and lack of literature on the subject, the minimal change in texture found by the PSA is inconclusive.

2.3.2 Bulk density and field capacity

Biochar had a significant effect on bulk density (ρ_b) in the 0-10cm depth. Table 2.3 shows that the plots with biochar alone had a significantly lower ρ_b compared to the control and effluent plots. Treatment B resulted in a ρ_b that was 7.3% less than that of Ct

and E. Those plots amended with both biochar and effluent, while eliciting a lesser bulk density than the plots Ct and E, did not produce significantly different results from each other, and the ρ_b of B+E was not significantly less than Ct or E.

Similarly, porosity was also affected by biochar amendment. Total porosity (f_T) was greater in both the biochar amended plots, but a significant effect was only found in treatment B compared to treatments Ct and E. The volumetric moisture content (Θ) and the f_T were calculated based on the gravimetric moisture content (w) and ρ_b , which were measured as described in section 2.2.5. However, the reported values in Tables 2.3 and 2.4 of w and Θ are not exactly proportional due to rounding.

Treatment	w %(g/g)	Θ %(cm/cm)	ρ_b (g/cm ³)	f_T (%)
Ct	6.0 ^a (0.4)	9.8 ^a (0.7)	1.50 ^a (0.07)	43.56 ^b (0.68)
B	7.0 ^a (0.3)	10.1 ^a (0.5)	1.39 ^b (0.03)	47.59 ^a (1.15)
E	7.0 ^a (0.1)	10.2 ^a (0.2)	1.50 ^a (0.01)	43.46 ^b (0.55)
B+E	7.0 ^a (0.2)	9.8 ^a (0.3)	1.45 ^{a,b} (0.02)	45.39 ^{a,b} (0.82)
LSD	1.0	1.5	0.07	2.56
p-value	0.92	0.92	0.01	0.01

Table 2.3. Soil gravimetric water content (w), soil volumetric water content (Θ), soil bulk density (ρ_b), and soil porosity (f_T); Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); $n = 4$ (aggregated from multiple locations on each plot)

Though porosity increased with the addition of biochar, the biochar did not significantly improve the water-holding capacity (WHC) of the soil. There were no significant differences in gravimetric water content among the treatments, and in fact, no indication of any trend indicating influence by biochar at the 0-10cm depth.

Treatment	w%(g/g)	Θ %(cm/cm)	Bulk density (g/cm ³)
C+F	6.0(0.4) ^a	9.6 ^a (0.6)	1.49 ^a (.03)
B+F	7.0(0.4) ^a	10.5 ^a (0.6)	1.45 ^a (.02)
LSD	1.5	2.3	0.10
p-value	0.32	0.32	0.28

Table 2.4. Soil gravimetric water content (w), soil volumetric water content (Θ), soil bulk density (ρ_b), and soil porosity (f_T); C+F-control+fertilizer, B+F-biochar+fertilizer ; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n = 3 (aggregated from multiple locations on each plot)

Furthermore, Table 2.4 displays the effects of biochar on the soil physical attributes of the conventional fertilizer amended plots. The difference in ρ_b is very similar to that of the effluent amended plots in the previous table - though B+F has a slightly lower bulk density, the effect is not significant. The water content at field capacity was also unaffected by biochar treatment.

Soil ρ_b changes with the addition of biochar are consistent with the results found by numerous other biochar studies (Killorn et al. 2010; Case et al. 2012; Zhang et al. 2011; Mikan and Abrams 1995). The reduction in ρ_b can be attributed to the higher porosity of the biochar in comparison to the soil, which also follows with most biochar studies. An anomaly present in this data though, is the lack of change in WHC between the biochar amended soils and their respective controls (Busscher et al. 2010). Kammann et al. (2011), for instance, calculated that WHC increased from 0.223g H₂O/g soil in the control plots to 0.276g H₂O/g soil in the 100Mg/ha biochar treated soil. The WHC also increased with the applications of 5 and 10Mg/ha of biochar in the study by Dugan et al. (2010). The discrepancy found between this study and others may be related to the

biochar production or application process. With regards to feedstock, Dugan et al. (2010) found that both saw dust and maize stover feedstock yielded an increased WHC, and Kammann et al. (2011) obtained similar results with peanut hull residues. However, the temperature of production in both studies was greater than 400°C. Though the HTT of the *Subabul* biochar used in this experiment is unknown, it is likely lower than the temperatures of pyrolysis that occur in a closed furnace, because it was produced in an open flame. Furthermore, Kamman et al. (2011) sieved the biochar to 2mm before application to soil, which increases surface area and possibly WHC.

Because the ρ_b of soil is increasing while w stays constant, it can be inferred that the pores introduced by the biochar are too large to measurably increase soil water retention. However, these results might not accurately depict the long-term effects of biochar on soil water relations, because as the biochar physically degrades the pore size distribution will change. A review by Lehmann and Joseph (2009) specifically explained that there is a positive correlation between biochar surface area and micropore volume. Furthermore, the presence of mineral-ash in biochar pores can occlude water from entering the pores, and the ash will eventually leach out as well. Therefore, there is potential for improved water retention by biochar over time.

2.3.3 Soil pH and EC

The pH of the soil was increased with biochar addition. Both B and B+E had a significantly higher pH, with B+E being less than B, than C and E. The effect of effluent on pH is uncertain. E had a greater pH than C, but the difference was not significant. This result reflects a common trend found in the literature. Biochar tends to have a pH above 7

(Lehmann and Joseph 2007), and the *subabul* biochar used in this experiment seemingly follows that trend and is greater than that of the local soil (Ct).

While the pH is an important element of soil quality (Lal 1994), the pH impact of biochar on this particular soil is apparently inconsequential to crop growth (as discussed in chapter 3) because of its initial neutrality (Bezdicek et al. 1996). In fact, a further increase in pH could lead to calcification, which would be detrimental to soil quality. However, B+E had a pH that was lower than B, but the change, again, was not significant (Figure 2.1). It is also important to note that these soil samples were taken one year after the amendments were applied, and thus leaching or volatilization could have diminished the pH effect of effluent. The effects of biochar, however, on soil pH are consistent with previous studies such as those reviewed by Jeffery et al. (2011), which found that on average biochar increases soil pH .6-1.0 units due to its alkaline nature. Similar results were also found by Chan et al. (2007), Sukartono et al. (2011), and Major et al. (2009). However, the ameliorating pH effect provided by biochar in these studies can be attributed to the soils' acidity. For instance, initial soil pH was reported as 5.97 in the sandy, volcanic soils studied by Sukartono et al. (2011) and 3.91 in the Savannah Oxisol (Major et al. 2009). In the present experiment though, there was no apparent increase in crop growth correlating to an increase in soil pH (see section 3.4.1-3.4.2). The response of this soil to biochar addition is also unlike the calcareous loamy soil tested by Zhang et al. (2011), who found that even a biochar of pH 10.5 was not able to affect overall soil pH.

Though the results are varied in the present study, the addition of biodigester effluent to soils often yields an increase in pH (Bachmann et al. 2011, Moller et al. 2008, Yu et al.

2010). Bachman et al. (2011) reported that the change in pH of neutral, loamy sand (similar to the soil in the present experiment) did not directly affect maize yield. Specifically, the greatest pH recorded resulted from the addition of cattle manure slurry – 7.12, and the lowest pH was 6.96, which was the result of NK addition. Though the pH was significantly different among treatments, the yields were not, just as is seen in the results of this experiment in relation to biochar addition (crop yields will be discussed in chapter 3).

Figure 2.1. Soil pH 1 year after treatment addition measured at a 1:1 soil:water ratio; Ct-control, B-biochar, E-effluent, B+E-biochar+effluent; SE=.07, .05, .06, .05, respectively; n = 4 (aggregated from multiple locations on each plot)

Figure 2.2. Soil EC 1 year after treatment addition measured at a 1:1 soil:water ratio; Ct-control, B-biochar, E-effluent, B+E-biochar+effluent; SE=25.64, 25.81, 38.38, 15.79, respectively; n = 4 (aggregated from multiple locations on each plot)

Soil EC was highest in treatment E and lowest in treatment B+E, and there was no clear pattern of the treatment effect on EC. EC values of C and B were between those of E and B+E with B being larger. Significance was found between E and B+E and E and Ct. As described previously, both biochar and biogas slurry do have the capacity to affect soil EC. The increase of EC in the E plots is similar to the results found by Yu et al. (2010). EC was not explicitly measured in most studies regarding the addition of biogas slurry to soil. However, effluent has been found to contain EC levels of 1.46dS/m and salt concentrations of 2.4gCa/L, for instance (Islam et al. 2010 and Gunnarson et al. 2010). Thus, in a non-saline soil such as the soil in the present experiment, addition of effluent could significantly increase soil EC. Additionally, experiments studying the effect of undigested manure on soil have found that undigested dairy slurry almost doubled EC

from 1415 $\mu\text{m}/\text{cm}$ to 2713 $\mu\text{m}/\text{cm}$ (Cameron et al. 1996). Li-Xian et al. (2007) also found that EC increased with successive applications of chicken and pigeon manure, increasing soil salinity from low to medium levels. However, Sommer and Husted (1995) assessed soil salinity after applying 17 different cattle, pig, and digested slurries, and discovered that most Ca^{2+} , Mg^{2+} , and PO_3^- were precipitated and not present in solution.

It is also unclear as to why the elevated EC was not maintained when the slurry was added in conjunction with biochar, and in fact, the EC of B+E plots was even lower than that of B plots alone. One explanation for this phenomenon is that the salts precipitated out of the solution or were immobilized to a greater extent when biochar and effluent were added together. Because biochar tends to have a negatively charged surface area when under alkaline conditions, it is possible that cations from the effluent were bound to the surface of biochar (Lehmann and Joseph 2009). If bound tightly enough to the biochar surface, the cations might not have been detected by the EC measurement.

2.3.4 Total N

Soil from t1 treatments were analyzed for their N contents one year after treatments were added to the plots. Table 2.5 presents the results of the N analysis. Differences in soil N content were not significant among treatments, demonstrating that there is little residual impact from the effluent addition in the previous year. This was expected in treatment E but numerous biochar studies have demonstrated its ability to retain nutrients (Knowles et al. 2010; Novak et al. 2009; Busscher et al. 2010; Laird et al. 2010), suggesting the biochar in this experiment would not reduce nutrient leaching.

Treatment	%N in soil
Ct	0.04(.002) ^a
B	0.04(.002) ^a
E	0.04(.001) ^a
B+E	0.04(.002) ^a
LSD	0.006
p-value	0.41

Table 2.5. %N in soil collected 1 year after initial application; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n = 4 (aggregated from multiple locations on each plot)

Though soil N was not analyzed in year 1, the significant improvements in crop yield in the first year most likely indicate that the effluent provided, to some extent, growth-limiting nutrients to the radishes (see chapter 3). As seen in treatment Ct, initial soil N content was very low; therefore, it can be inferred that one of the contributions provided by the effluent in year 1 was plant available N.

The effects of biochar on soil N in this experiment diverge from the studies that suggest that biochar can increase the availability of nutrient fertilizer. Knowles et al. (2011) demonstrated the effect of Monterey Pine (*Pinus radiata*) biochar on nitrate leaching in two silt loam soils over a 6-month period. The biochar was added along with biosolids, which were applied at rates of 600 kg N/ha and 1200 kg N/ha. Though the total volume of leachate was equivalent among treatments, when biochar was added in addition to biosolids, nitrate leaching was reduced significantly. The total nitrogen leached per hectare was, for instance, 36 kg/ha in the Ashley Dene silt loam (ASL) soil at the 600 biosolids rate+biochar and 163 kg/ha in the ASL soil at the 600 biosolids rate without biochar. It is unclear, however, the mechanism through which biochar limited nitrate

leaching. Biochar CEC was less than that of either soil, anion exchange capacity was also low (these 4.0 cmol(+)/kg), and a lack of differences in leachate volume suggest that biochar pores did not play a role in inhibiting nitrate transmission through the soil profile. Thus, Knowles et al. (2011) proposed that toxic agents on the surface of the biochar suppressed nitrogen mineralization and nitrification, implying that these results are largely dependent on the biochar feedstock, which was, of course, different from that which was used in the present experiment.

Laird et al. (2010) tested a hardwood biochar in a similar fashion during a 45-week rainfall simulation study on 15kg soil columns. In the soil column with swine manure alone total N leached was 186 mg, and increasing additions of biochar of 5, 10, and 25g/kg decreased nitrogen leaching to 175, 167, and 165 mg, respectively. The latter 2 quantities are significantly less than the N leached from the manure alone. The impact of biochar on soil nitrogen status is not completely straightforward, however. The biochar+manure treatments also only decreased NO₃-N leaching at the 5 and 10g/kg biochar rates while the 20g/kg rate actually increased NO₃-N leaching compared to the manure only treatment. This may be due to enhanced microbial mineralization of organic N caused by biochar addition. It should also be noted that biochar addition in the absence of swine manure did not result in a consistent reduction of NO₃-N leaching with increasing rates of biochar. Laird et al. (2010) explained that biochar likely adsorbed NH₄⁺ and soluble organic compounds from the manure, this apparently did not occur in the non-manure amended treatments. The results in Laird et al. (2010) are also similar to those seen in Steiner et al. (2007) and Steiner et al. (2008)

Numerous other studies that corroborate the conclusion that biochar improves nutrient retention in soils do not necessarily extend that conclusion to include soil N. For instance, Novak et al. (2009) found that the addition of pecan biochar to a loamy soil enhanced soil concentrations of P, Ca, K, and C, but actually reduced soil N content from 1.24g/kg to 1.21g/kg. The same is largely true for the 1st year in the study by Major et al. (2009). Furthermore, in that same study, Major et al. (2009) also observed a decrease in total soil N between the 1st and 2nd year even with the addition of biochar and subsequent fertilization treatments. Therefore, perhaps an analysis soil N alone is not sufficient to address the question of whether the *subabul* biochar can improve soil quality in the long-term through increased nutrient retention.

Finally, another important factor to consider is the N content of the biochar itself. The N content of biochar can range from as little as 1.7 g/kg N to greater than 70g/kg N (Lehmann and Joseph 2009). The N content of the biochar in this experiment is given in Table 2.6, and it is comparatively small. Because these total N analyses were performed on 1 aggregated biochar sample, it cannot be determined whether there is a significant difference between the %N of the biochar and the biochar+effluent. However, according to this analysis, the biochar plus effluent does have a slightly greater N content, suggesting a minute amount of effluent N was retained by the biochar.

Sample type	N%	C%	C:N ratio
Biochar	0.69	70.15	102.2
Biochar+Effluent	0.71	72.91	102.6

Table 2.6 C and N content of biochar hand-sampled from plots 1 year after application; n=1 (aggregated from multiple locations on multiple plots)

2.4 Conclusion

The texture of the soil on which this experiment was conducted was loamy sand. Biochar reduced soil ρ_b and increased soil f_T . However, biochar did not have an effect on soil WHC, implying that the additional porespace contributed by biochar was too large to retain water. Literature suggests that over time microporosity might increase, but long-term, field biochar studies must be conducted to confirm that hypothesis.

Analysis of soil samples collected 1 year after treatment application suggest that biochar increased soil pH but that the effluent had no effect on it. Soil EC was significantly increased by effluent alone, but the impact was not seen when effluent was added in conjunction with biochar, indicating that salts from the effluent might have bound to the biochar, reducing the measurable EC. Because soil N was not significantly greater in the B+E compared to E and B, it can be inferred that the biochar utilized in this experiment was not able to retain N over the course of the 15 month experimental period. However, a more extensive nutrient analysis must be performed to address whether the biochar retained other plant essential nutrients. Furthermore, if an increased microporosity is developed over time, that too might allow for improved nutrient retention.

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3 Biochar and anaerobic digester effluent impact on crop growth

Abstract

Biochar has been proposed as a means of making water and nutrients more plant available, and anaerobic digester effluent has been studied as a locally producible substitute for conventional fertilizer. Utilizing these amendments also provide a means for mitigation of climate change and other localized environmental challenges. A fifteen-month experiment was conducted to evaluate the effects of biochar and effluent on crop growth. In year 1, biochar alone (B) did not significantly increase crop yields, but effluent alone (E) did compared to the control (Ct), producing yields that were 25 times greater than Ct. Biochar and effluent together (B+E) further improved crop yields, indicating an interactive effect between the two amendments. Plant height, length, number of leaves, and above- and below-ground yields were measured in year 1. During the 2nd year, there were no significant differences in leaf elongation rate, LAI, yield, or leaf N content among treatments. In the second year, two new treatments were introduced – conventional fertilizer and conventional fertilizer+biochar. These treatments also did not significantly differ in the aforementioned parameters. The results of this experiment suggest that biodigester effluent is an effective fertilizer and that interaction with biochar can produce compounding, positive results. However, this biochar was not able to make nutrients from the effluent plant available 1 year after application, and its interactive effect with conventional fertilizer seems more tenuous.

3.1 Introduction

3.1.1 The effects of biochar addition on crop growth

Studies on the effects of biochar on crop growth have generally found positive results.

For instance, Chan et al. (2008) added biochar manufactured at 450°C to a C-deficient

Alfisol to grow radish, and an addition of 10Mg/ha and 50Mg/ha of biochar alone increased total dry matter (TDM) yield by 42% and 96%, respectively. Biochar (50Mg/ha) added in conjunction with N fertilizer increased yield 320%. Many studies, however, have found that the sole addition of biochar does not yield as promising results as it does when added along with nutrient fertilizer. This is largely because most agricultural soils are N-limited (Vitousek et al. 1997), and biochar alone does not generally address that limitation. Biochar, as described previously, typically has a high C:N ratio. It is not likely, however, that biochar exacerbates the challenge of N availability by immobilizing N because its chemical and biological decomposition rate is very slow (Novak et al. 2009).

The interaction between mineral fertilizer and biochar is complex and dependent on biochar, soil, and crop types, but the interaction yields largely positive results. Two biochars, both produced from sludge and waste wood chips, added to a Ferrosol in combination with mineral fertilizer produced wheat yields of 1.4 and 1.2g per pot in comparison to the biochar alone, which produced yields of 0.4 and 0.5g per pot. Comparable results were also seen for soybeans (*Glycine max*) and radish grown during the same experiment (Van Zwieten et al. 2009). The benefits of N fertilizer addition were also seen in a study by Zhang et al. (2010) in which wheat straw biochar added at 20Mg/ha on loamy soils produced a 15.8% increase in maize yield from 6.28Mg/ha in the control to 7.27Mg/ha in the biochar plots, whereas biochar added with N fertilizer (7.86Mg/ha) led to an 18.2% increase crop yield compared to N fertilizer alone (6.65Mg/ha).

As seen in the Zhang et al. (2010) study, biochar has at times yielded

supplemental effects in addition to the benefits contributed by the mineral fertilizer.

According to a study by Rondon et al. (2006) in which wood biochar was added to Typic Haplustox at 0.8 and 20Mg/ha, biochar added in conjunction with conventional fertilizer increased yields by up to 30% compared to fertilizer alone. Similar results were found when chicken manure and city waste biochars were added to a silt loam soil, which produced maize yields of 3.34 Mg/ha and 3.30Mg/ha, respectively, in the second year. These were significant yield increases of between 38 and 40% compared to the control (Utomo et al. 2012). This supplemental effect was also demonstrated in an Indonesian acid soil – 15Mg/ha of bark biochar was added with and without mineral fertilizer, and the yield from the biochar+fertilizer treatment was approximately 50% greater than that of the fertilizer treatment alone (Yamato et al. 2006).

Application of biochar does not always increase crop yields even in the presence of fertilizer, however. When 3Mg/ha of wood biochar and 120kg N/ha were added to a silt loam soil, the yield produced was both less than the fertilizer alone as well as the control (Yeboah et al. 2009). When Knowles et al. (2011) added a pine wood biochar to silt loam soils in New Zealand, ryegrass (*Lolium perenne* Bronsyn) yielded a similar result. In fact, pine wood biochar either reduced or negated the yield improvements made by both the biosolids fertilizer as well as the conventional fertilizer tested during the experiment. The authors proposed that the biochar could have retained the nutrients such that they were inaccessible to plants or contained a toxic substance.

One mechanism through which biochar improves crop growth, however, is by retaining nutrients in a region of the soil that is plant accessible and preventing leaching. A study on a Columbian Oxisol by Major et al. (2009) found that the greatest nutrient benefit

provided through wood biochar amendment was increased available K present in the biochar itself. This result reflects a common trend of reduced nutrient leaching (Lehmann et al. 2003; Rondon et al. 2006), but in all cases the increased nutrient availability is limited to the first year or cropping season. The wood biochar also increased availability of Ca and Mg that were added in the form of dolomite because it prevented the nutrients from leaching, an expected occurrence in Oxisol soils. This fertilizer absorption was maintained throughout the four-year experiment. While the mineral fertilizer interaction has been demonstrated frequently, it is not wholly consistent. Graber et al. (2010) added NPK fertilizer along with 1, 3, and 5Mg/ha of biochar in a pot experiment. During the experiment, plant available nutrients did not increase significantly.

In the experiment by Van Zwieten et al. (2010) the increase in crop yield was also attributed to the rise in pH with the addition of biochar, which increased up to 1.73 units from 4.20 in the control to 5.93 in the sludge and wood waste biochar-amended soils.

Additionally, both biochar amendments tested during the Van Zwieten et al. (2010) experiment increased soil CEC of the Ferrosol, which was an effect not seen in the study by Major et al. (2009). It is important to note that biochar impact on soil CEC increases over time (Lehmann 2007), however, so more data needs to be collected on long-term biochar experiments to properly assess the ability of biochar to impact soil CEC.

According to Van Zwieten et al. (2010), wheat had an increased N uptake efficiency in the experiment, which was attributed to the improved rooting environment that resulted from the compound effects of the increased pH and CEC. This phenomenon was also observed by Chan et al. (2007). Just as is the case with other biochar effects though, an

increase in soil pH and CEC does not invariably occur (Zhang et al. 2011; Knowles et al. 2011).

As explained previously, biochar has the potential to reduce ρ_b and increase water retention, and numerous studies have demonstrated the subsequent improvement in plant growth caused by these changes in soil characteristics (Glaser et al. 2002). One experiment amended a sandy soil with cow dung-based biochar at 3 different rates (10, 15, 20Mg/ha) to assess biochar effects on maize growth. The two larger amendment rates significantly enhanced crop growth. A portion of the improved yield was attributed to the 139 and 91% increase in water use efficiency attained by maize in the 15 and 20Mg/ha rates, respectively (Uzoma et al. 2011). Kammann et al. (2011) also witnessed an increase in plant water use efficiency (WUE) due to peanut (*Arachis hypogaeae*) hull biochar addition on a sandy soil. Specifically, the biochar allowed for increased accumulation of osmotically active species in the quinoa (*Chenopodium quinoa*) tissue, stimulating water uptake. Furthermore, biochar addition also corresponded with enhanced root biomass, making water plant available in the pores of the biochar. Though quinoa grown in biochar-amended soils produced increased leaf area, the plants also were observed to have a lower transpiration rate, further improving WUE. Quinoa biomass increased with both biochar addition rates, 100 and 200Mg/ha, in comparison to the control.

A fifth mechanism through which biochar can positively impact crop growth is through improved soil structure. These results were demonstrated, for example, when bagasse- and biosolids-based biochars were added to a clay soil in Japan. Both biochars increased sugarcane (*Saccharum officinarum*) growth, which can be explained by both a significant

increase in soil moisture and other alterations in chemical composition as well as a decrease in soil ρ_b . Sugarcane roots were thicker and longer in the plots with the reduced ρ_b (Chen et al. 2011). Another experiment performed on a hardsetting Alfisol amended with greenwaste biochar concluded that tensile strength increased with increasing rates of biochar, partly influencing the augmented radish growth seen on the biochar-amended plots (Chan et al. 2008).

Finally, biochar has been associated with microbial interactions, which are conducive to plant growth. For example, positive yield responses seen in Yamato et al. (2006) were partially attributed to augmented mycorrhizal colonization of plant roots. Conversely, when tomato (*Lycopersicum esculentum* Mill.) and pepper (*Capsicum annum* L.) plants were grown in soil along with a wood biochar, improved plant growth was ascribed to the decrease in susceptibility to two foliar pathogens among the biochar-amended soils (Graber et al. 2010).

While the chemical, physical, and biological effects of biochars on soils often give rise to positive results on crop productivity, there are also studies in which biochar addition has had little or no impact on crop growth.

3.1.2 The effects of biodigester effluent on crop growth

Biodigester effluent has been studied for its efficacy as a fertilizer for several decades. In fact, biodigesters are generally accepted as a technology that can be utilized to improve crop growth while reducing the environmental impact of untreated livestock manure (Ayre and Callway 2005). Studies of biodigester effluent have largely focused on its ability to improve nutrient availability to plants and thus crop yields (Yu et al. 2010; Gunnarsson et al. 2010; Lundvall et al. 2007; Bachmann et al. 2011). However, effluent

impacts on crop growth, while overwhelmingly beneficial, have varying mechanisms and degrees of success.

Dahiya and Vasudevan (1986) assessed the effects of cattle dung-based effluent addition on 9 field crops and found that effluent-amended soils produced greater yields than the control in all cases. Specifically, the effluent treatment led to an 82.3% yield increase (2.06kg/plot) in wheat, 81.4% increase (0.27kg/plot) in mustard (*Brassica campestris*), and 53.0% increase (10.02kg/plot) in cauliflower (*Brassica oleracea*) versus the control (1.13kg/plot, 0.27kg/plt, and 10.02kg/plot, respectively). However, the authors also found that in vegetable crops the highest yields were attained when effluent was added in combination with mineral fertilizer (each fertilizer contributes 50% of added N) while fodder crops produced the best results when amended with effluent alone. An effluent-based fertilizer (in whole or part) was most successful in improving plant growth even in comparison to a 100% mineral fertilizer. The mineral fertilizer alone was more effective, however, in 4 of the 9 plants.

A similar experiment was performed by Bachmann (2010) testing a cattle dung effluent, a codigested effluent (including cow dung, maize silage, and wheat grain), and two types of mineral fertilizers on sandy and loam soils. Maize production in both soils was greater with the addition of either effluent than with NK fertilizer. However, NPK fertilizer produced significantly higher maize yields in the sandy soil compared to the effluent, which was not apparent in the loam soils.

Some studies have suggested that effluent can provide nutrients in a more plant available form than undigested cow dung because a larger portion of the N will exist as ammonium (Gertsson et al. 2004). For instance, Rubaek et al. (1996) found that total ammonium-N

loss for digested effluent was only 37% while undigested effluent losses were 47%, significantly higher. In fact, one study even found that biogas slurry and concentrated biogas slurry increased plant available N, P, and K more than conventional fertilizer (Yu et al. 2010).

However, the evidence supporting that conclusion is uncertain. Moller et al. (2008) found that digested effluent did not substantially increase the supply of N to plants compared to manure or undigested slurry over the long-term. However, the digested effluent produced significantly higher winter wheat yield than the undigested treatments. A similar study comparing digested and undigested swine manure indicated similar results – crop available N was not changed significantly due to digestion. Both effluents were added such that total N applied was equal at any given rate, and the study concluded that yield was dependent on N application rate instead of the source (Lundvall et al. 2007).

The extent of effluent effect on crop growth could also depend on soil type. For example, one study added both cow dung and codigested (mixed feedstock) effluent to sand and loam soils. On loam soils both forms of effluent had an equal impact on maize yields, which was significantly higher than conventional NPK treatment, whereas the NPK treatment performed significantly better than both effluents, which remained equal, on the sandy soil (Bachmann et al. 2011).

Though anaerobically digested effluent is not consistently as successful a soil amendment as conventional fertilizers, it consistently improves yield compared to unfertilized soil. Gunnarson et al. (2010) added 75 and 150mg N/dm² in the form of biodigester effluent (with grass and clover feedstocks), and even at the lower rate, the effluent produced

significantly higher ryegrass yields than the unfertilized plots. Similar results were found consistently among papers reporting anaerobic digester effluents from various sources.

3.2 Experimental design

Refer to section 2.2.1.

3.3 Materials and methods

3.3.1 Field measurements of plant growth

In year 1, observations of number of leaves and leaf length were taken on day 20 and day 40 of growth following planting. Plant height was a measure of the longest leaf of each selected plant. Eight plants were randomly chosen from each plot as the sample for both measurements on both days.

Furthermore, in year 2 leaf elongation rate was recorded. The 4th true leaf of 4 randomly chosen plants on each plot was followed over 9 days. Each leaf's length was measured on days 1, 2, 3, 4, 6, 7, and 9, and growth rates were compared across treatments. Due to herbivory, some data, especially those from the NPK-amended plots, were excluded.

During the second year, leaf area index (LAI) was also calculated. LAI photographs were taken on the 18th day of radish growth. Two photographs of three randomly selected plants were taken from each plot and analyzed with Photoshop CS6, using the procedure described in section 3.3.3.

3.3.2 Leaf elongation calculation

For each leaf, $\ln(\text{leaf length})$ was regressed against time during the last 4 days of the 9 day period. To compare the slopes of the regression equations, an ANOVA was performed among treatments to produce the mean exponential growth rates for each treatment and their respective stand errors.

3.3.3 LAI calculation

The leaves in field photographs were digitally separated from the remaining image using the Color Range tool. The histogram tool was used to calculate the number of pixels in an object of known area also in the photograph. The same tool was used to calculate the number of pixels covered by the selected leaves in the photograph. Finally, leaf area was evaluated using the area of the object, its number of pixels, and the number of pixels contained in the leaves.

3.3.4 Crop yield

Crops were harvested on the 54th day after planting in the first year. Fresh weight was taken immediately after harvest, after which plants were divided into roots and shoots. The inflorescence had already developed before harvest, so flower parts were further separated from leaf material. A sample of ten whole radish plants from each plot was sun-dried (at approximately 50°C) to a constant weight, and a final dry weight for the plot was extrapolated from the sample by using the percent water in the sample and the fresh weight measured at harvest. The aggregated dry weight from each plot was utilized for the ANOVA among treatments. The same process was followed for the harvested plants in year 2. However, the final harvest occurred on day 20 due to rabbit damage.

3.3.5 Leaf nutrient analysis

Dried leaves from the final harvest were ground and passed through a 50µm sieve. .500g of the powdered leaf tissue was homogenized on the SampleTek Vial Rotator and analyzed using a CN Analyzer (Vario Max, Elementar Analysensysteme, Hanau, Germany).

3.3.6 Statistical Analysis

Analyses of variance for most crop and leaf growth data were conducted utilizing the PROC GLM procedure in Statistical Analysis Software (SAS) 9.2. Least Significant Difference (LSD) values were also calculated using the same procedure. The leaf elongation rate was analyzed using the PROC MIXED procedure (SAS Institute, 2008). The results from year 1 are based data from 7 replications of each t1 treatment. Results from year 2 are based on 4 replications of each of those treatments, and when indicated, 3 replicates of t2 treatments. The graphs were produced using STATA 12.1 (STATA Corp., 2011).

3.4 Results and discussion

3.4.1 Crop yield year 1

On the 20th day after planting, there were already stark differences among the effluent and non-effluent treatments. As indicated by Table 3.1, both E and B+E had significantly greater leaf lengths than Ct and B. This same pattern was reflected in number of leaves as well. Additionally, there was no difference between the B plots and their respective controls. It is clear that none of these plant growth changes were due to a difference in competition among plants, because the stand count performed on day 20 confirms that all of the treatments had approximately 47 to 48 plants with no significant differences among treatments.

Treatment	Height (cm)	# of Leaves	Number of Plants
Ct	4.2 ^b (0.2)	4.2 ^b (0.1)	47.6 ^a (0.2)
B	4.1 ^b (0.1)	4.2 ^b (0.1)	47.4 ^a (0.4)
E	8.3 ^a (0.2)	6.2 ^a (0.1)	47.6 ^a (0.2)
B+E	8.4 ^a (0.4)	5.9 ^a (0.2)	47.9 ^a (0.1)
LSD	0.67	0.33	-
p-value	<0.0001	<0.0001	0.66

Table 3.1. Plant growth, 20 days after sowing; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); For height and # of leaves n = 56

After 20 more days, a second set of field measurements were taken (Table 3.2). While the E and B+E treated plants grew on average 120% and 143%, respectively during that period, the leaves of treatments Ct and B only increased 38% and 87%, respectively. Furthermore, treatments B+E began to diverge from E, producing significantly longer leaves, suggesting there was a compounding effect when biochar was added along with effluent.

The leaf count demonstrates a similar pattern among treatments, where E treatments have greater numbers of leaves than treatments Ct and B. However, when comparing the number of leaves, there was no demonstrable difference between the E and B+E treatments. On day 40, plants in the B treatment begin to diverge slightly from the plants in Ct. Treatment B plants were consistently larger and had a greater number of leaves than the Ct treatment unlike during the previous measurement. Furthermore, for both measurements, the differences between B and Ct are very close to being significant.

Treatment	Height (cm)	# of Leaves
Ct	5.8 ^c (.06)	6.0 ^b (0.5)
B	7.6 ^c (0.6)	6.7 ^b (0.5)
E	18.2 ^b (0.7)	10.4 ^a (0.2)
B+E	20.5 ^a (0.9)	10.5 ^a (0.3)
LSD	2.1	1.2
p-value	<0.0001	<0.0001

Table 3.2. Plant growth 40 days after sowing; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n = 56

Table 3.3 and Figure 3.1 display final dry weights and stand counts of each treatment.

Though the stand count was equal on day 20, over the course of the field season

treatments Ct and B experienced some mortality while treatments E and B+E largely maintained the a consistent stand count until the day of harvest. Differences in stand

count were reflective of other measures of plant productivity as well. Total dry matter

(TDM) comparisons among treatments followed the same trend as stand count, for

instance. Effluent addition significantly increased TDM yield as well as the above and

below ground biomass (including the hypocotyl) yield, separately. TDM for Ct plots was

31.29g and TDM for E plots was 412.98g. Biochar additions had a less substantial effect

though. B and B+E had a TDM yields of 59.73g and 461.31g, respectively. There was no

significant difference between treatments Ct and B in tuber yield, above ground biomass,

or in dry weight. That was also the case with regards to E and B+E. It should also be

noted, though, that above ground biomass yields of the B+E treatment is very close to

being significantly greater in mass than the E treatment, and B+E throughout the growing period has had consistently greater yields than treatment E.

Treatment	Below ground biomass (g)	Above ground biomass (g)	Stand Count
Ct	2.0 ^b (0.5)	4.4 ^b (1.0)	43.4 ^b (1.5)
B	2.8 ^b (1.5)	7.9 ^b (2.7)	43.4 ^b (1.5)
E	43.5 ^a (5.8)	26.0 ^a (3.7)	46.7 ^a (0.3)
B+E	51.5 ^a (4.3)	32.0 ^a (3.0)	47.1 ^a (0.4)
LSD	10.7	8.05	3.15
p-value	<0.0001	<0.0001	0.03

Table 3.3 Year 1 above and below ground dry mass of 10 randomly selected plants – final harvest; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); For biomass n=7

Figure 3.1. Year 1 total dry matter yield 54 days after sowing; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; SE=6.4, 21.7, 44.1, 42.6, respectively; n = 7

The impact of biodigester effluent on crop growth is directionally in accordance with other studies on the agronomic value of effluent. However, it seemed to perform better than most effluent treatments, compared to the control, than has been seen in other papers. During this experiment, adding effluent increased TDM from 31.29g to 461.31g, which is more than a 10-fold increase in dry matter yield, whereas in the study by Dahiya and Vasudevan (1986), cattle manure-based biodigester effluent only improved cauliflower (*brassica oleracea*), a member of the same family as radish, growth by 88.8%. The yield difference between the control and liquid cow-dung-based biogas slurry in Laura and Idnani (1972) was slightly greater, a 112% increase from 25.1g of wheat to 52.1g of wheat, respectively. However, it is still an order of magnitude smaller than the increases found during this experiment. A third experiment, again, found modest increases of wheat grain yield – from 4.40Mg/ha in the control to 6.21Mg/ha in the effluent plots (Garg et al. 2005).

There are two possible explanations for this discrepancy. The major explanation is a difference in initial soil fertility between the plots in this experiment and other experimental plots. As mentioned in the site description, the land on which these radishes were grown had not been cultivated in at least 10 years. In addition, they are sandy soils from which nutrients can leach very quickly. Whereas, the experiment by Dahiya and Vasudevan was conducted on a loam soil with a 30.4% water holding capacity that had been utilized for experiments in the past. Similarly, the soil medium in the Laura and Idnani paper was clayey in texture and contained .11%N before effluent additions. Finally, Garg et al. (2005) added NPK to all of the plots in the experiment, regardless of

treatment. This is likely the explanation for the vastly different responses to biogas slurry application.

A second explanation, however, is that the rate of effluent application differs significantly. The rates of application of slurry cannot be compared precisely among experiments because in two of the studies slurry application was reported by weight, whereas in this experiment slurry was applied by volume, and the exact density of the biogas slurry added in this experiment is unknown. However, literature suggests that biogas slurry is approximately 90% water (Laura and Idnani 1972), so conservatively estimating that density of the slurry is 1.0g/mL, application rate would be approximately 56.25Mg/ha. This rate is about three times larger than the rate applied by Garg et al. (2005) and five times larger than the rate applied by Laura and Idnani (1972). Since the effluents likely contained a growth-limiting nutrient, perhaps a small increase in application rate could lead to vastly larger plant growth. It should also be noted that the experiment conducted by Laura and Idnani (1972) was a pot experiment in which 8 plants were growth per pot, thus decreasing the potential nutrient loss and competition. Nevertheless, as stated previously, the increase in plant growth with the addition of effluent is a result supported by literature.

In the first year, biochar addition alone increased TDM yields by about 91% compared to the control (though the result was not significant). Biochar addition with effluent increased TDM yields by approximately 12% compared to effluent alone, which was also not significant. These non-significant yield effects are very much in accordance with the literature. A meta-analysis of 177 biochar studies found that overall biochar induces a 10% increase in crop yield with less positive effects shown in field studies than

greenhouse studies. Specifically, a comparison among 142 replicates of radish plots, there were statistically significant increases in crop productivity with the addition of biochar, whereas 7 of the other 10 crops studied showed no significant effect (Jeffery et al. 2011). Thus, a largely non-significant, but positive growth response by a radish crop in a field experiment follows the trend suggested by this meta-analysis.

Van Zweiten et al. (2010) also demonstrated modest and often non-significant yield increases with biochar amendments. Biochar produced from enhanced solids reduction sludge and wood chips at 550°C increased radish and soybean growth compared to the control from approximately 1.25g to 1.5g and .25g to .5g on a ferrosol soil. The same biochar did significantly increase soybean yield when combined with N fertilizer, compared to the N fertilizer plots. However, a second biochar (made from slightly different ratios of the same materials) studied in the same experiment did not produce significantly greater yield even when considering the N fertilizer interaction.

Similar results were produced by Yeboah et al. (2009) in which 3Mg/ha of biochar added to a sandy loam soil increased maize root and shoot yield by a total of 1.74g compared to the control, and the LSD was 4.46g. In the same soil 3Mg/ha of biochar+120kg N/ha yielded a 4.70g increase in crop yield compared to the control, a slightly significant increase. The same biochar produced consistent but non-significant negative crop yield results compared to the control when tested on a silt loam soil. In both cases, P and K fertilizer were added to all treatments. A third experiment also found concurrent results – a slight but non-significant maize increase when comparing chicken manure biochar+N fertilizer to N fertilizer alone (Utomo et al. 2012).

Positive yield increases that are not analogous to the results in these experiments have been demonstrated as well. For instance, in the experiment by Chan et al. (2007) dry matter yield of radish increased from 1.95g/pot in the N fertilizer only plots to 3.72g/pot in the 50Mg/ha chicken manure biochar+N fertilizer treatment and 4.48g/pot in the 100Mg/ha biochar+N fertilizer treatment, which are both significant yield increases that can be directly attributed to biochar addition. However, the increased significance could be explained by biochar rate, because when 10Mg/ha of chicken manure biochar was added to the pots, there was no significant yield increase. The yield increase was only seen at a 50Mg/ha biochar rate. Furthermore, biochar type and, possibly, temperature vary between this experiment and the experiment conducted by Chan et al. (2007).

With equal fertilization, Uzoma et al. (2011) determined that 15Mg/ha of cow manure biochar added to a sandy soil significantly increased maize height compared to the control beginning 6 days after germination and continued that trend throughout the growing period. Final yield in the biochar-amended plot was 150% that of the control plot. In the Uzoma et al. (2011) experiment, explanations for improved growth due to the addition of biochar include the resulting increase in pH from 6.40 to 7.34, increase soil C and N contents, and an increase in CEC. Though soil pH did increase in the present experiment, like results were not seen. Any C and N changes due to biochar addition also did not affect crop growth (discussed in section 3.4.3). Though the *subabul* biochar utilized in this experiment did appear to produce sufficiently less positive results than those by Chan et al. (2007) and Uzoma et al. (2011), these results should be considered in light of the observation by Jeffery et al. (2011), which stated that pot experiments on biochar tend to produce three times greater increases in crop productivity than field trials.

3.4.2 Leaf growth year 2

A comparison of the leaf elongation rates among t1 treatments shows that there were no significant differences among leaf growth rates of each treatment (Table 3.4). The consistently larger productivity from the addition of effluent seen in year 1 was not continued in year 2. This was even the case in the B+E treatment, which based on previous literature (Busscher et al. 2010; Knowles et al. 2011; Laird et al. 2010; Major et al. 2009) was expected to aid the soil in retaining the nutrients during the intermediary year. Compared to Ct, treatment B also did not continue to display the slightly greater productivity that was witnessed in year 1.

Treatment	Exp. Growth Rate (ln(cm)/d)
Ct	0.5 (0.1) ^a
B	0.4 (0.1) ^a
E	0.3 (0.8) ^a
B+E	0.3 (0.1) ^a
p-value	0.41

Table 3.4. Year 2 leaf elongation rate; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n(Ct) = 8, n(B) = 4, n(E) = 12, n(B+E) = 9

Treatment	Exp. Growth rate (ln(cm)/d)
C+F	0.1(0.02) ^a
B+F	0.1(0.02) ^a
p-value	0.06

Table 3.5. Year 2 leaf elongation rate; C+F-control+fertilizer; B+F-

biochar+fertilizer; within columns values followed by the same letter are not significantly different; Reported as mean(SE); $n(C+F) = 12$, $n(B+F) = 11$

Table 3.5 illustrates the leaf elongation rates of the NPK amended plots. It should be noted that the growth rates in Table 3.5 cannot be directly compared with those from Table 3.4 because the data in Table 3.5 were collected at a later period of leaf development than Table 3.4, and according to Cutler et al. (1980), the rate of elongation declines as the leaf matures. However, just as the previous table demonstrated, biochar did not increase the rate of growth in comparison to the control even when combined with conventional fertilizer. In fact, plant growth in treatment B+F was slightly slower than that of C+F plants, with the C+F treatment almost being significantly greater than the B+F treatment.

Leaf area index (LAI), similar to leaf elongation rate, also did not vary among treatments in year 2 (Table 3.6). As expected, the LAIs of the t2 plots were greater than the t1 plots (Table 3.7). Though the LAI was not calculated in year one, measurements such as aboveground biomass is indicative of the variations that might have been seen in LAI. Thus, the lack of significant differences among t1 treatments in the second year implies that biochar did not retain a sufficient amount of nutrients from the effluent added initially to enhance crop growth in the second year. With regards to the inorganic fertilizer treatment comparison, the non-significant differences in LAI also indicate that the biochar did not provide a supplemental effect on leaf growth.

Treatment	LAI
Ct	0.01 ^a (0.002)
B	0.01 ^a (0.001)
E	0.02 ^a (0.004)
B+E	0.01 ^a (0.000)
LSD	0.006
p-value	0.28

Table 3.6. Year 2, leaf area index (LAI); Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n = 8

Treatment	LAI
C+F	0.06 ^a (0.01)
B+F	0.06 ^a (0.01)
LSD	0.04
p-value	0.995

Table 3.7. Year 2, leaf area index (LAI); C+F-control+fertilizer; B+F-biochar+fertilizer; within columns values followed by the same letter are not significantly different; Reported as mean(SE); n = 6

Both LAI and leaf elongation rate are related to nutrient and water intake(Chapin et al. 1988; Bunce 1977; Tanguilig et al. 1987; Vos et al. 2005; Steponkus et al. 1980). In this experiment, water input was controlled. However, throughout the experiment among all treatments there were visible signs of water stress. Bunce (1977) determined that there was a linear relationship between leaf elongation rate and turgor pressure, which is in turn directly related to leaf water potentials. Steponkus et al. (1980) corroborated these results in an experiment, which tested the responses of various rice cultivars to water stress. Though plants conditioned for drought tolerance were less sensitive to reduced

water availability, once a threshold level was reached (-1000 to -1400 kPa) leaf elongation ceased in these plants as well.

The effect of water stress on the LAI of radish seems less clear, however. Wan and Kang (2005) studied the effect of irrigation treatments on radish LAI and found, in the 1st year that the least frequent irrigation treatments, once every 8 days and once every 6 days, resulted in LAIs that were equal to that of more frequent irrigation treatments. In the 2nd year, however, they resulted in the 2nd and 3rd lowest LAIs of all the treatments, respectively. Kang and Wan (2005) similarly found that lower soil water potentials did not cause a reduction in LAI. In fact, year 1 results in the Kang and Wan (2005) study found an inverse relationship between soil water potential and LAI as low as -55 kPa. Carmichael et al. (2012) also studied the effects of moisture availability on radish growth, specifically observing the impact of various mulching mediums. However, the Carmichael et al. (2012) study found that LAI was positively correlated with increased moisture availability. Water was applied whenever soil matric potential reached -1000 kPa, and the 3 moisture levels tested were 5.0, 3.5, 2.5 L/pot. Because all of the treatments received an equal amount of water at the same times of day in the present experiment, it logically follows that leaf elongation rate and LAI are not significantly different among treatments. However, N that was potentially provided by the effluent or inorganic fertilizer and enhanced in the presence of biochar can also have an impact on LAI and elongation. Table 3.8, again, suggests that these impacts did not take place, because there were no significant differences found in leaf N content among T1 treatments. T2 treatments were not included in this analysis.

Treatment	Total N (%)
Ct	4.39 ^a (.090)
B	4.32 ^a (.135)
E	4.30 ^a (.121)
B+E	4.10 ^a (.084)
p-value	0.276

Table 3.8. Year 2 Total N in leaf tissue 20 days after planting; Ct-control; B-biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE)

Because the N content in leaves sampled were normal, and even slightly high, for a c3 plant (Brown 1978) it can be determined that N was not the major growth limiting factor in this soil. One explanation for the high tissue N concentrations could be the stunted growth of the plants. As will be seen in section 3.2.3, the plants analyzed in Table 3.5 are low-mass plants, which tend to have greater N contents than larger plant at the stage of growth. The decreased N concentration is due to an increase in the plants' structural and storage tissue that have low N contents (Greenwood et al. 1990). In sum, the N data presented above suggests that water or an alternate nutrient, not N, is responsible for restricting the growth of t1 plants.

Another explanation for the low LAI and leaf elongation rate in all the t1 treatments could be a P limitation. Plénet, et al. (2000) found that LAI and, to a greater extent, elongation rate are both affected by P availability. When 0 kg/ha/yr of P was added to the soil, the resulting LAI was consistently, significantly less than that of the 42.8 kg/ha/yr treatment and the 94.3 kg/ha/yr treatment, which were generally not significantly different from each other. The leaf elongation rate was significantly lower in the 0 kg/ha/yr treatment compared to the 42.8 kg/ha/yr treatment for leaves 5-8, but the difference was no longer significant for leaves 9–12. Especially because the radishes in

this experiment were harvested on day 20 and most plants had only 5 or less leaves at the time of harvest, P deficiency could also have played a major role in limiting leaf growth. Furthermore, as will be described in the next section, the crop growth of NPK amended plots was approximately 4 times that of all t1 treatments, and because all other factors were controlled, it is likely that either P or K was a growth limiting nutrient. If a P deficiency was, in fact, present in the B+E treatment in year 2 that further signifies that biochar did not store the P, which if present in the effluent, contributed to yield increases the previous year.

3.4.3 Crop yield, year 2

Table 3.9 displays the stand count taken 7 days after planting in order to confirm that plant competition did not confound the treatment effect. Though initial stand count did not differ among treatments, variations in TDM among treatments were also not seen. Dry weight was measured for 24 individuals (the entire stand was not harvested) at day 20, and the TDM was between 2 and 3g for all treatments. There is a clear contrast between the treatment effects in year 1 and the treatment effects in the second year, indicating that the impact of the effluent on crop growth did not persist into the second year even with the addition of biochar.

Treatment	Initial stand count	Total dry matter (g)
Ct	47.5 ^a (0.3)	2.5 ^a (0.3)
B	47.8 ^a (0.3)	2.4 ^a (0.2)
E	47.0 ^a (0.4)	3.0 ^a (0.2)
B+E	47.5 ^a (0.5)	2.5 ^a (0.1)
LSD	1.2	0.9
p-value	0.60	0.50

Table 3.9. Year 2 stand count and total dry matter yield 20 days after planting; Ct-control; B-

biochar; E-effluent; B+E-biochar+effluent; within columns values followed by the same letter are not significantly different; Reported as mean(SE)

Treatment	Initial stand count	Total dry matter (g)
C+F	44.3(1.7) ^a	10.1(0.2) ^a
B+F	46.7(0.9) ^a	8.3(0.7) ^a
LSD	5.2	2.0
p-value	0.28	0.06

Table 3.10. Year 2 stand count 7 days after planting and day 20 total dry matter yield; C+F-control+fertilizer; B+F-biochar+fertilizer; within columns values followed by the same letter are not significantly different; Reported as mean(SE)

TDM in the t2 plots were between 4 and 5 times greater than the plots with t1 treatments. The results from Table 3.10 also diverge slightly from the results seen in year 1, because the TDM in the B+F treatment is less than in the C+F treatment, almost to a significant extent. Similarly, the B+F treatment grew slower than the C+F treatment, according to the leaf elongation rate measurement. However, when E was compared with B+E in year 1, slightly higher (but mostly non-significant) yields were present in the treatments with biochar compared to the treatments without biochar. This comparison confounds the trend suggested in the year 1 data – that biochar slightly increases crop growth, especially when added in conjunction with a nutrient fertilizer, which is a trend also seen in the literature (Steiner et al. 2007; Major et al. 2009; Kimetu et al. 2008). One explanation for this phenomenon could simply be the time of measurement. As seen in year 1, the divergence in growth between E and B+E increases over time, but the data available in year 2 are only from the first 20 days of growth. Therefore, it is possible that the growth rate of treatment B+F would have been higher later in the growing period, allowing it to equal or surpass the TDM yield of C+F at a later time.

The data from year 1 and year 2 must be compared with caution, however, because the radish varieties grown were slightly different – *Pusa Chetki* in year 1 and *Pusa Chetki Long* in year 2. Other environmental and treatment factors varied between the years as well, including temperature and watering rate.

As mentioned previously, there is very limited information on the long-term effects on biochar on crop growth. In both the studies by Major et al. (2010) and Steiner et al. (2007) the effect of biochar on crop yield increased with time. After the second year, Major et al. (2010) found that the biochar+NPK fertilizer treatment produced a maize yield, which was 28% greater than the control (approximately 5Mg/ha). In year 3, the yield was 30% greater than the control, which produced about 5.9Mg/ha. Finally, after 4 years, the biochar+fertilizer treatment was 120% that of the control (approximately 1.8Mg/ha). One explanation given for the long-term benefit of biochar to crop growth in the experiment is an increased availability of Ca and Mg due to a reduction in leaching. Leaching is also expected to be a problem in the present experiment, because the soils were determined to be loamy sand, but there is no evidence that the biochar was able to prevent the leaching of water and thus biochar is unlikely to have reduced nutrient leaching this experiment. This may explain the discrepancy in biochar impacts in the present experiment and the experiment by Major et al. (2009). The production techniques of both biochars are unknown, however, so it is difficult to assess the precise reasons for the varied crop responses.

Steiner et al. (2007) also found a synergistic effect between biochar and inorganic fertilizers in all four harvests of rice (*Oryza Sativa L.*) and sorghum (*Sorghum biocolor L.*) on a Xanthic Ferralsol, which enhanced grain yield and stover production compared

to the mineral fertilizer amendment alone. Biochar had a greater synergistic effect in year 2 than in year 1. In fact, grain yield increased by a factor of 9 compared to mineral fertilizer alone, which produced approximately .1Mg/ha. Again, the effect witnessed by Steiner et al. (2007) was not replicated in the present experiment. One explanation for improved yield was, as seen previously, a reduced soil acidity, which was not necessary in the present experiment because the Lakkihalli soils were neutral. Another explanation, however, is an increased soil WHC contributed by the biochar. The second harvest, in which Steiner found the greatest yield difference between the biochar+mineral fertilizer and mineral fertilizer alone, was the driest season, which suggests that the biochar was able to improve yields through the increase in water retention. Though the increase in WHC is expected to be, in fact, more prevalent in sandy soils (Glaser et al. 2002), it did not occur in the Lakkihalli experiment.

3.5 Conclusion

Biodigester effluent had a consistently positive impact on crop growth and yield in year 1. There are some data providing evidence that biochar further improved growth, but final TDM yield was not significantly affected by biochar addition. Year 2 leaf growth data, including elongation rate and LAI, consistently indicated no significant differences among t1 treatments. This was reinforced by day 20 yield data from the same growing period. T2 treatments also did not produce significantly different yields or leaf elongation rates. Finally, t1 treatments did not have a significant impact on leaf tissue N in year 2.

Because of an incomplete chemical analysis of both the soil and plant tissue, it cannot be stated with certainty that the lack of differences in plant growth among t1 treatments in the 2nd year are attributed to a limited water availability or a P deficiency specifically, but the

vastly contrasting yields among the E and B+E treatments in year 1 and 2 indicate that some component of the effluent – be it water or a plant essential nutrient – that was made available in year 1 was no longer present in year 2. Furthermore, the B+F treatment did not significantly increase crop yield or leaf size compared to C+F, suggesting that those nutrients were either not made more bioavailable by the biochar or that NPK were no longer the limiting factors in leaf growth in those treatments.

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4 Research and policy implications

4.1 Summary of conclusions

After the completion of the 15-month study, the impacts of a single biochar and biodigester effluent application on soil quality were found to be minimal. Biochar decreased soil ρ_b and increased f_T . Biodigester effluent increased EC, but the effect was diminished when the effluent was added in conjunction with biochar. Soil N was unchanged with all treatments 1 year after their addition.

During the first year, radish growth was significantly improved with the addition of biodigester effluent. Biochar also slightly, but non-significantly, increased radish growth throughout the season. These positive results did not extend into year 2. No t1 treatment increased crop growth significantly compared to the control, and none of the treatments contained statistically different quantities of plant tissue N. Year 2 conventional fertilizer treatments produced greater crop yields than the t1 treatments, but there was no evidence that biochar-treated plots further improved crop yield significantly.

4.2 Implementation challenges

Conducting an experiment in a developing country and coordinating with a local NGO (BIRD-K) proved to be exceptionally challenging. The three major barriers to implementation of this project were disorganization and, at times, lack of cooperation by NGO workers; lack of availability or failure of seemingly vital equipment and facilities; and Indian government bureaucracy. Apart from these relatively controllable factors, herbivory by rabbits and insects also complicated the data collection process.

Beginning at the initial stages of the planning process, the BIRD-K contact was somewhat deceptive regarding the facilities available locally. For instance, she claimed that a biogasifer was present on-site to produce biochar. However, after arrival, it was discovered that the biogasifer had not been functioning for several months, and there was no way of repairing it before the start of the project. In addition, even before arrival, *subabul* trees (not tree branches as we had agreed upon) were cut and burned in a traditional fire to produce the experimental biochar. This is an unsustainable method of biochar production and somewhat defeated the premise of the experiment – to test the successfulness of an environmentally sound agricultural management system.

Incompetence and noncompliance continued throughout the experiment. BIRD-K attempted to irrigate the field with a sprinkler system that did not evenly water every plot even after explicit instructions were given to the contrary. Furthermore, the fence, which was initially built around the field during the 1st cropping season, was removed without permission after the season to be used for other NGO projects, ultimately resulting in field disturbance by cows and sheep.

Projects in rural India also must be resilient to electricity and water shortages. Rain-fed agriculture is unheard of between January and March in Karnataka, and therefore the radishes had to be irrigated. However, the only water available for irrigation was tens of meters below the ground – the average borewell depth in India is well over 1000 feet, according to Solomon (2010) – and had to be pumped up with a generator, which ran on electricity. There was no electricity during the daytime however, and occasionally outages would last for several days at a time, making it difficult to water the crops. By the second year, a faro-cement tank was obtained to efficiently collect the water during the night and store it until it

was needed. That tank was in high-demand, however, and on numerous occasions other NGO workers attempted to abscond with the tank.

During year 1, some soil and plant analyses were performed in Bangalore in collaboration with the University of Agricultural Sciences (UAS) and Anatech laboratories. The experiments on soil physical properties were largely unsuccessful because of poor laboratory conditions and inadequate facilities. Again, power outages made 24-hour oven drying nearly impossible. Sieves and accurate balances were few in number and inaccessible due to overcrowded laboratories and rules governing non-students. Anatech laboratories performed a chemical analysis on year 1 soil and plant samples, but the results were highly variable and seemed completely inaccurate, for instance suggesting C:N ratios in plants that were essentially impossible.

Therefore, in order to ensure reliability of results, the second year samples were sent to the U.S. for analysis. Mailing plant and soil samples out of the country was also incredibly challenging though. Most Indian government bureaucrats consulted during this experience that worked in the state government Horticulture Department, the agency that is supposed to provide phytosanitary certificates for the goods, are not actually aware of the Indian government regulations regarding the export of agricultural goods. They either entirely deny any requests for said certificate or take advantage of their and others' ignorance by creating additional obstacles (at an additional expense) to receiving the certificate. Ultimately, after two months of research and communications with various government officials and shipping companies, the plant and soil samples were successfully sent to the U.S.

An accurate field experiment requires consistency in process, avoidance of contamination, timeliness, and numerous other well-controlled factors, both environmental and human.

Many of these requirements are difficult to meet even in state-of-the-art facilities and well-managed experimental plots, but they are infinitely more difficult in a remote village in India, a country that actively creates challenges for non-citizens performing research.

4.3 Discussion on policy and research

The experiment in Lakkihalli and others discussed throughout this paper afford some policy suggestions as well as numerous avenues for further research. Firstly, as discussed in previous sections, there are barriers to even sustainably producing biochar and anaerobic digester effluent for use on agricultural fields. Feedstocks for these materials, such as animal manure and crop residues, are often used for cooking fuel and household upkeep. Both gasifiers and digesters are not presently available in all Indian villages, and the facilities often go into disrepair soon after introduction into a village, as was the case in BIRD-K (the biodigester fell into disrepair soon after the end of season 1). Successful employment of biochar and biodigester effluent as agricultural amendments will require government programs that not only install digesters and gasifiers in Indian villages but somehow provide maintenance services for those facilities. Additionally, it must be clear to Indian farmers that conversion of biomass into biochar and effluent is the most effective use of their resources. While the results from year 1 and other studies suggest that biodigester effluent is, in fact, an efficient, effective method of leveraging the energy and fertilizer potential of community biomass resources, the results suggest biochar is not. This experiments suggests that, if used by local farmers, effluent must be applied more frequently than once a year. Whether nutrients from the effluent leached through the soil profile or volatilized, the effluent added in year 1 was no longer available to plants in year 2. Biochar, however, has had inconsistent results with regards to nutrient and water retention in several of the experiments discussed

throughout the paper, and many positive results are obtained from the addition of impractically large amounts of biochar. Therefore, before the large-scale deployment of biogasifiers in India or elsewhere, substantial research is necessary to improve the scientific community's understanding of the effects of feedstock and temperature on the physical and chemical characteristics of biochar, which dictate its effectiveness as a soil amendment. Furthermore, there is a dearth of long-term, field experiments on biochar, which provide the most realistic assessments of its efficacy.

One possible avenue for incentivizing the wide-scale adoption of either biochar or biodigester effluent is a carbon valuation scheme. If utilizing biomass to produce these soil amendments is not immediately the most cost-effective option for rural farmers, then carbon offset payments could make these practices more economically viable. Generally, direct combustion of organic materials, even for cooking is not as sustainable or efficient as producing biogas and effluent or biochar and syngas, which are both fuel and fertilizer and create fewer GHG emissions per unit energy produced. The effluent also reduces the need for conventional fertilizers, which are petroleum-based products. The emissions reductions produced from increased water and fertilizer efficiency due to biochar addition are less certain however. Infrastructure to verify or make payments for these emissions are not at all in place nationally or internationally, although there are NGOs attempting to leverage existing offsets and C trading schemes to promote sustainable agriculture. These NGO projects can essentially act as pilot studies to determine whether the adoption of biochar and biodigester effluent can produce real, verifiable emissions reductions and improved crop yields. These pilot studies could also act as the aforementioned long-term biochar field studies. While this experiment did not wholly affirm the long-term benefits of either soil

amendment, the results suggest that anaerobic digester effluent is a promising substitute for conventional fertilizer and that the large-scale adoption of biochar would be premature without additional research.

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