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Assessing Kiln-Produced Hardwood Biochar for Improving Soil Health in a Temperate Climate Agricultural Soil

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ASSESSING KILN-PRODUCED HARDWOOD BIOCHAR FOR IMPROVING SOIL HEALTH IN A TEMPERATE CLIMATE AGRICULTURAL SOIL

A Dissertation Presented

by

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DEDICATION

For my grandfather, Donald L. Morris, who was a great man. He made all who knew him richer in life and love.

For my parents, Mike and Linda, who nurtured my independent spirit and love of getting dirty.

&

For my husband and best friend, Ray. Thank you for the love, the constant support, and for going first.

ABSTRACT

ASSESSING KILN-PRODUCED HARDWOOD BIOCHAR FOR IMPROVING SOIL HEALTH IN A TEMPERATE CLIMATE AGRICULTURAL SOIL

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Soil quality has become a major factor used in assessing sustainable land management and the overall environmental quality, food security, and economic viability of agricultural lands. Recently, biochar has been touted as having many potential uses as a soil amendment for improving soil quality, specifically improving cation exchange capacity, pH and nutrient availability. However, soil biology also plays a significant role in biogeochemical processes that influence soil health and should be included in a more comprehensive study of soil health. This dissertation describes 4 projects within the same 3-year field study with the cumulative purpose of better understanding the effect that the application of a hardwood biochar has on soil health and quality. (1) The evaluation of a hardwood lump charcoal production by-product for use as a quality biochar amendment,

tested the hypothesis that this byproduct has physical and chemical properties that fall within the range of quality biochar amendments as proposed by recent literature.   (2) A three-year assessment of the changes to the soil chemical and physical properties as affected by the addition of the hardwood biochar to agricultural soils. Biochar application caused so significant differences in sweet corn yield or quality. (3) A three-year assessment of the yield of sweet corn grown in the same biochar amended soils with and without added nitrogen fertilization also showed that a two percent by weight application of hardwood biochar improved yields of sweet corn, but greater application rates had a negative priming effect. Thus, testing the hypothesis that biochar would improve agronomic yields. (4) In year three of this field study, nematode community assemblages were identified and compared in both the control and highest biochar treatments. Nematode communities were significantly different with maturity indices indicating greater temporal stability in the biochar-amended soils. (5) The bacterial diversity of the control, two percent and four percent biochar-amended field soils were assessed using high-throughput sequencing of 16S rRNA and taxonomic assessment. While overall community diversity was not significantly affected, the abundance of specific bacterial taxa were significantly affected, indicating the potential for shifts in biogeochemical cycling in biochar-amended soils.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The sustainability of small farms in the Northeastern U.S. has been continually threatened by both the rising costs to operate and this continued degradation of soil quality. The number of small farms (sales less than $50,000 per year) in operation fell 7.7% from 2002-2007 (USDA-NASS, 2009) and another 3% from 2007-2012 (USDA-NASS, 2014) and has been declining for several decades. This has a significant affect on the agricultural market, as small farms currently comprise 75% of the total US farms. Small farms are constantly competing with large farms for economic viability. In fact, large farms are becoming even more profitable (USDA-NASS 2014), and without the comparably large land area to increase profitability, small farms must find alternate ways to increase yield and/or decrease operating costs to stay profitable. One large factor involved in the sustainability and yield is the quality and health of the soils. Soil quality must be maintained to ensure high crop yield, yet often, conventional farming practices cause continual soil quality degradation from intensive cultivation and inorganic fertilizer application. Counteracting this inverse relationship is a major challenge and often requires significant shifts in agricultural management practices.

Anthropogenic degradation of the world's agricultural lands has surpassed 40% due to over-use and poor management practices and concerns should rival the concern associated with major ecological issues like global climate change and loss of global biodiversity (Oldeman, 1994; Doran and Zeiss, 2000). Soil quality, as defined by the Soil Science Society of America, is the "capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Karlen et al., 1997). Soil

quality has become a major factor used in assessing sustainable land management and the overall environmental quality, food security, and economic viability of agricultural lands. In fact sustainable agriculture and increasing soil quality have become intricately related and practically synonymous (Herrick, 2000). Although soil health or quality is a complicated network of relationships that are not easily defined, soil quality degradation is most often described in terms of organic matter depletion, compaction, poor water holding capacity, and low biological activity and defined by Oldeman (1994) as the process "which lowers the current and/or future capacity of soils to produce goods or service." This is a broad definition that encompasses chemical degradation, physical degradation and erosion.

In a 1999 opinion article, Sojka and Upchurch discuss the difficulty of setting parameters and qualifiers to determine a soil quality assessment scale (Sojak Upchurch, 1999). Both water and air quality can be measured using predetermined chemical analysis tests that do not take into account the use of that air or water and the environment surrounding the sampling location. To truly determine soil quality is a much more difficult task. Soils can naturally acquire chemicals that may be considered toxic simply sue to their geographic location and soil forming processes. On top of that there are twelve very distinct soil orders that vary in their chemical and physical characteristics greatly, even without anthropogenic influence.

Continuously cropped lands lose significant amounts of soil organic matter every year. Targeting the loss of soil organic matter (SOM) can be a highly effective and practical method of increasing soil quality and thus improving soil fertility especially in continuously tilled cropping systems (Reeves, 1997). The addition of compost or manure can significantly increase SOM content and specifically the soil organic carbon (SOC). However, the increase is often temporary as biological degradation of SOM to carbon dioxide occurs quickly. Even

with best conservation tillage practices there are still significant losses in SOM from mineralization unless significant amounts of biomass are returned to the soil (Glaser, 2007). Maintaining adequate levels of SOM is essential for maintenance of soil health and sustainable agricultural practices (Reeves, 1997) that are necessary to combat the world-wide decrease in agricultural production per person that is currently occurring. The decrease in agricultural production and lower soil quality is especially alarming as the world's population increases. Increased storage or reduce loss of nutrients, like nitrogen, in and from the soil will decrease the need for fertilizer application and increase the overall sustainability and efficiency of agricultural systems around the globe.

Recently, there has been a surge in research on increasing the soil quality through a specific type of soil amendment. This amendment has been termed "biochar" (Lehmann et al., 2003a) as it is a charcoal-like carbon substance that is specifically being used in the soil environment and research. Originally discovered in the Amazon Basin and referred to as Amazonian Dark Earths, or Terra Preta *de Indio* soils (Glaser et al., 2000; Smith, 1980), biochar has been touted as having many potential uses as a soil amendment including remediation of contaminated soils, carbon sequestration and specific soil characteristic alteration, such as increased cation exchange capacity, pH and nutrient availability (Ding et al., 2010; Graber et al., 2010; Hollister et al., 2013; Laird et al., 2010; Lehmann et al., 2003a; Rajkovich et al., 2011; Schomberg et al., 2012).

Although there have been many positive findings regarding the increased soil health of biochar-amended soils, the findings mostly highlight the increase in health of highly degraded soils. Studies that focus on the effects of temperate soils with moderate health are few and far between. Significant changes in an extreme soil with biochar are more likely, yet many agricultural soils are found in less extreme climates than the Amazon Basin.

The high application rates of nitrogen fertilizer to agricultural lands causes or contributes to environmental hazards such as nitrate contamination of groundwater and eutrophication of surface water eco-systems (Camargo and Alonso, 2006). High demands on crop production keep nitrogen fertilization as an essential component of food production, so in order to alleviate the problem of nitrate contamination, it must be held in the soil instead of so easily leached away. One possible action to reduce leaching and aid in nitrate retention is through the addition of biochar to soils (Lehmann et al., 2003a). The potential increase in soil organic carbon and water holding capacity may aid in retention of nitrogen in the form of nitrate in the soil.

Studies have shown enhanced yield of rice, up to 220%, with application of biochar in highly weathered soils (Glaser et al., 2002; Lehmann et al., 2003a). Biochar may dramatically improve the chemical, physical and biological properties of these highly weathered soils, but the potential is present to also ameliorate such properties in less weathered soils, such as the temperate soils local to this research study (Glaser, 2007). While the potential for improvement is present, a drastic improvement, such as those found in the tropics, is not expected in this project. However, some increase in marketable yield is expected in future years. Studies have also shown that additional fertilization is often essential for high productivity and increases the positive effects of biochar on crop yield (Lehmann and Rondon, 2005).

CHAPTER 2

LITERATURE REVIEW

2.1 Terra Preta

Although black carbon has been applied to soils for hundreds (if not thousands) of years and there is documented research dating back to the 19th century, this recent up swell of research brings with it a new set of challenges and questions to answer. The discovery of Terra Preta *de Indio* (Indian Black earth) soils in the Amazon is possibly the largest factor that spurred the recent surge in biochar research and its affect on agricultural soils. These Terra Preta soils were discovered in the 1950's but it was not until Nigel Smith published "Anthrosols and Human Carrying Capacity in Amazonia" in 1980, that these soils were argued to be of anthropogenic origin. Most of these Terra Preta soils contained pottery shards, and thus have linked to early human settlements in the Amazon since their discovery. Prior to this, they were not believed to be of anthropogenic origin. One early theory on the development of these fertile soils suggested that these 'Dark Earth' regions were developed from volcanic ash deposits or another theory suggested that those Terra Preta sites near rivers were from lake bottom deposits and that local people happened upon these soils and settled there specifically to utilize their increased fertility to support agriculture (Smith, 1980). These soils are characterized by deep A horizons (40-80 cm) which are significantly darker than neighboring soil (Glaser et al., 2000), yet they are of the same soil texture (Zech et al., 1990). This leads researchers to believe that the Terra Preta soils were somehow derived from the local soils (Glaser et al., 2001).

Figure 1: Soil Profiles (a) Terra Preta soil and (b) nearby Oxisol soil. This image is adapted from Glaser et al., 2001.

Using samples of Terra Preta soils from several sites in regions near to rivers and the flood plains of the Amazon basin as well as in regions unlikely to flood, Smith looked deeper into the soil characteristics to find clues to their origin. They are regions of varying sizes dotted throughout and surrounded by relatively infertile Oxisols and Ultisols (Smith, 1980). In contrast to the local infertile soils, the Terra Preta soils have remained fertile for hundreds of years despite intense leaching, humid conditions, high temperatures, limited stabilizing minerals, and a high amount of precipitation, which can cause rapid organic matter mineralization rates and result in lower efficiency of organic fertilizer when applied in these humid tropic soils (Glaser et al., 2001; Glaser et al., 2002). This distinct increase in fertility is one of the indicators of anthropogenic origin. Several key nutrients that are typically lacking in other Amazonian soils are phosphorus, calcium and nitrogen. Terra Preta soils, across the board, have significantly higher levels of these nutrients. Nigel Smith's research focused on phosphorus as a key indicator of human influence on the

development of these soils. Phosphorus has long been found in soils that are of anthropogenic origin. It is likely present in the Terra Preta soils due to the incorporation of fish bones, urine, turtle shells and fecal matter deposited by local communities that were settled in one place for a significant amount of time. Over time, as these communities burned their refuse, phosphorus and other nutrients were released into the soils along with the charred biomass that remained (Smith, 1980).

This charred biomass apparently caused yet another difference and increased fertility in Terra Preta soils as compared with other local soils. The Terra Preta soils contain up to three times the amount of soil SOM and 70 times the amount of black carbon than other soils in the region (Glaser et al., 2001; Glaser, 2007). Charred biomass when burned in piles allows limited oxygen exposure to the internal biomass, and forms benzene-like carboxylic acid structures. These structures do not result from typical microbial decomposition or humification processes. Also, the charred residues of slash and burn practices do not contain a highly stable carbon fraction as is found in Terra Preta soils. The largely aromatic carbon present leads to the discovery that the cause of increased black carbon content in the Terra Preta soils is the continued anthropogenic charring of plant biomass and then incorporation of that charred material into the A Horizon (Glaser et al., 2000; Glaser, 2007).

The black carbon is highly aromatic which is attributed to the partial condensation of lignin during the incomplete combustion of woody tissues (Glaser, 2001). This high amount of aromaticity contributes significantly to the nutrient holding capacity especially as the black carbon becomes oxidized (Glaser et al., 2000). This same aromaticity also leads to the increased stability of the black carbon as well as increased porosity from the original plant biomass to the charred product. Radio carbon dating has found black carbon in the

Amazon region as old as 7000 years (Neves et al., 2001). The long-term stability means long term benefits for the Amazonian soils. The black carbon causes greater stabilization of organic matter with Terra Preta soils containing up to three times as much SOM than un-amended neighboring soils, causing slower release of nutrients and better retention of cations. These factors contribute to overall higher crop production (as much as double) than the nearby Oxisols. These Terra Preta soils are so fertile that they have been excavated and sold for over 30 years (Smith, 1980).

2.2 Manufacturing Terra Preta: Biochar

Bapat and Manahan (1998) first used the term biochar to refer to the charred product of pyrolysis of biomass, and the term has come to imply use for carbon sequestration and soil amelioration. Biochar is a carbon-enriched, porous material produced at high temperatures from an organic feedstock. It is different from charcoal, which also can result from a low-oxygenated burn, as the direct purpose of biochar production is to add it to the soil and charcoal most often used for energy. Biochar is also different from activated carbon, as it has not been treated to increase the surface area with steam or in any other manner.

2.2.1 Pyrolysis

Biochar is generally heated in a sealed container with little or no oxygen. This process is referred to as pyrolysis. As a result of pyrolysis, the biochar contains a high level of aromatic organic carbon (Keiluweit et al., 2010), similar in structure to the black carbon found in Terra Preta soils, which can then be used as a soil amendment. Interestingly, also as a result of the pyrolysis, there is a net negative atmospheric carbon effect. Through

pyrolysis, the carbon in organic matter is rendered stable and relatively inert. Black carbon does not readily react with oxygen nor is it easily metabolized by soil microorganisms, thus the carbon leaves the atmospheric carbon cycle and instead enters the slower geological carbon cycle (Forbeset al., 2006; Lehmann, 2007). It is because of this negative carbon process, that biochar has continued to gain interest for its many possible uses. Were pyrolysis not a carbon negative process as compared to the natural carbon cycle, biochar would likely have gained little momentum or interest over the past decade. In the natural carbon cycle, 100% of the carbon from a living plant would eventually return to the atmosphere as carbon dioxide through the processes of decomposition. Approximately 20-25% of the carbon from the living biomass will potentially be kept from returning to the atmosphere as carbon dioxide if it undergoes pyrolysis instead of natural decomposition (Lehmann, 2007). It will remain in its highly stable, largely aromatic form with a mean residence time (MRT) of centuries, if not millennia (Kuzyakovet al., 2014) all the time increasing the soil health due to organic matter stabilization, slower nutrient release, increased CEC and water holding capacity (Lehmann and Rondon, 2005).
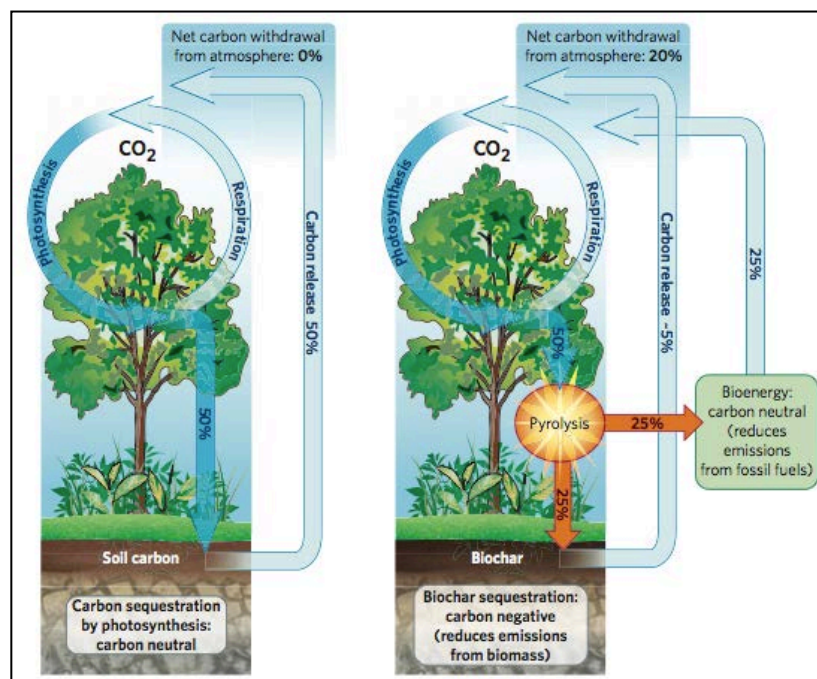
Figure 2: Diagram of carbon negative pyrolysis process. This image is adapted from Lehmann, 2007.

Pyrolysis temperature and procedure as well as the biomass chosen as feedstock all influence the characteristics of the biochar produced. To generalize characteristics across all biochars produced would be wildly inaccurate and would mask the many nuances involved in determining whether a biochar application would benefit a specific soil. The work of Dr. Novak and others highlights the vast differences in biochars produced through difference circumstances in "Characterization of Designer Biochar Produced at Different Temperatures and their Effects on a Loamy Sand" (Novak et al., 2009a). In this work, several different starting materials were used to produce biochar- peanut hulls, pecan shells, poultry litter, and switch grass and though all were produced using a slow pyrolysis process, several different temperatures of pyrolysis were used as well. The temperature of pyrolysis plays a significant role in the final biochar structure. The organic starting material is composed of a variety of compounds, primarily cellulose, hemi-cellulose, and lignin

compounds. These compounds begin to degrade at different temperatures and all play a part in the final biochar structure (Downey et al., 2009).

Economically, biochar production can be a profitable side effect from traditional charcoal production. Over 41 million tons of charcoal is produced annually worldwide. When charcoal particles do not meet the size requirement for sale, they can be sold as biochar for soil amendment purposes (Glaser, 2007). This increases the profitability of charcoal manufacturers, as well as increasing the efficiency of their process. However, there is still doubt that large-scale biochar production can become a profitable market mainly due to the uncertainty involved in the carbon sequestration market (Roberts et al., 2010). Potential mainly lies in utilizing on-farm waste, such as crop residue, animal litter and/or manure and yard waste, and local or on-site pyrolysis to produce biochar affordably for agricultural uses (McHenry, 2009).

2.3 Properties of Biochar

2.3.1 Structural changes

The structure of biochar is mostly that of highly aromatic carbon rings with significant stability. As the original biomass is pyrolyzed near-edge X-ray adsorption fine structure spectroscopy (NEXAFS), as well as many NMR studies, shows an increase in aromatic carbon-carbon bonds and a significant decrease in single carbon-carbon bonds (Keiluweit et al., 2010). NEXAFS is a type of absorption spectroscopy that uses high-energy wavelength of light to investigate electron energy levels of core atoms in solid states. It is useful to characterize biochar in this manner as it can look at trends in the electron energies of carbon atoms in the biochar and Terra Preta soil samples (Liang et al., 2006). Energies of

aromatic and non-aromatic carbon atoms are distinctly different. Comparing the NEXAFS of biochar samples to graphene (which is entirely aromatic) can even aid in determining in the amount of aromaticity in each biochar sample. Increasing aromaticity gives the biochar structure greater stability and more resistance to biological degradation creating the long-lasting effects seen in the Terra Preta soils. NEXAFS spectroscopy also shows that there is a significant loss in functional groups as the feedstock is pyrolyzed into biochar. The structure becomes increasingly more carbon-based and other elements such as hydrogen oxygen and nitrogen are off-gassed as bio-oil or syn-gas during pyrolysis. The final structures of biochar products vary, but all have some degree of turbostratic graphene arrangement. This means that the graphene sheets are not highly ordered and aligned as they are in graphite or diamond. The planar sheets have larger spacing than graphite, which gives the biochars a range of pore sizes (Downey et al., 2009).

DRIFT (diffuse reflectance infrared Fourier transform) spectroscopy is also very useful in determining the chemistry of biochars. DRIFT spectroscopy can also analyze the increasingly carbonic structure of biochar. The starting biomass often contains highly acidic or negative functional groups such as carboxylic acids and hydroxyl groups. Reports of aliphatic alcohols and acid functional groups disappearing from DRIFT spectra as the pyrolysis temperature increases have been thoroughly investigated and reported (Mukherjee et al., 2011). These negative functional groups would potentially increase the cation exchange capacity of the biochar if they were to remain on the surface of the biochar after pyrolysis.

Though the large majority of carbon in biochar is composed of condensed aromatic moieties (Kuvyakov et al., 2014) there can still be a significant amount of other organic compounds. Lipids, polysaccharides, and other easily mineralized compounds can undergo

somewhat fast decomposition. Within 1-2 years, however, the remaining carbon is mainly in aromatic structures that are not easily decomposed by soil biota and are recalcitrant in the soil (Schimmelpfennig and Glaser, 2012). In fact, Kuyakov et al. (2014) found mean residence time in temperate climates as high as 4000 years, with slow rates of decomposition as low as 0.26% per year, over a 9-year study. This two-speed carbon mineralization, (fast then slow) supports that longer-term studies are necessary to fully understand the stability of biochar in the soil.

2.3.2 Elemental Composition

Historically, elemental compositions have been used to describe various characteristics of both coal and charcoal (Schimmelpfennig and Glaser, 2012). As pyrolysis most often results in the loss of significant amounts of hydrogen, oxygen, and even methyl groups (Amonette and Joseph, 2009), the resulting elemental composition tends to results in higher percentages of aromatic carbon as structures condense and other elements are lost during the heating process (Baldock and Smernik, 2002). There are various spectroscopic methods available that can be used to approximate the aromatic carbon percentage of the resulting carbon structure as mentioned in the previous section. However, it may not be necessary to turn to spectroscopic methods to analyze the stability of the resulting biochar. Van Krevelen diagrams have been used for decades to compare the hydrogen to carbon ratio (H/C) and the oxygen to carbon ratio (O/C) in carbon materials (van Krevelen, 1957). These elemental ratios can also be used to assess the degree of carbonization in biochars (Schimmelpfennig and Glaser, 2012).
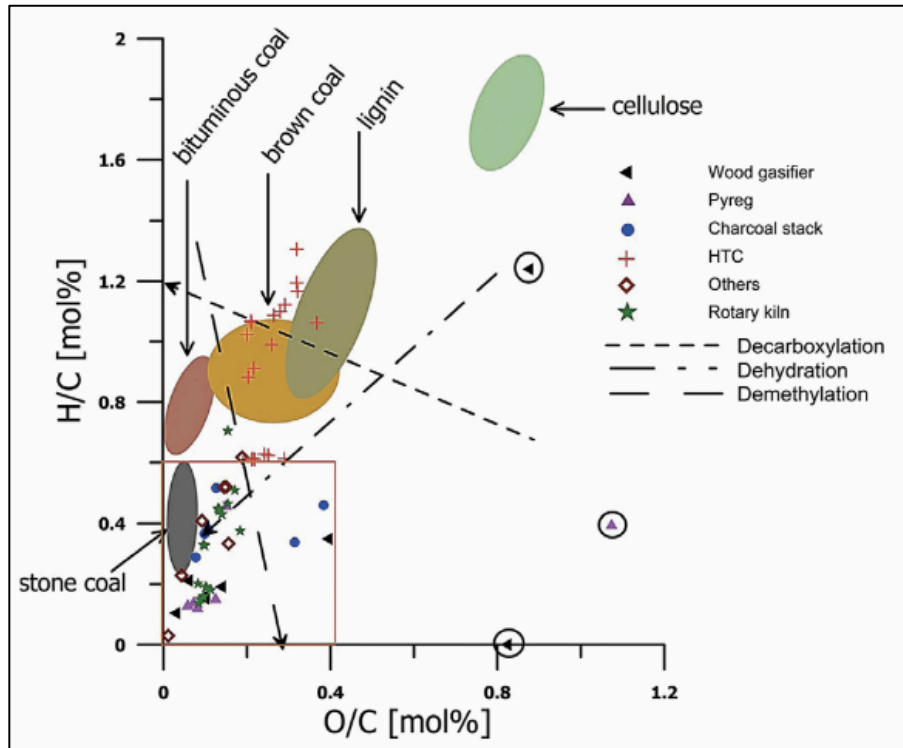
Figure 3: Van Krevelen diagram of both plant material and the product of pyrolysis of biomass by numerous methods (adapted from Schimmlepfennig and Glaser, 2012).

2.3.3 Cation Exchange Capacity

As the surface charge of a biochar becomes more negative through oxidative processes, the cation exchange capacity (CEC) increases. Considering that cations are positive, this makes sense as electrostatic attraction between the negative biochar surface functional groups and the cations increase. However, the cation exchange capacity varies significantly based upon both the feedstock and the pyrolysis temperature. Mukherjee et al. (2011) found that low temperature grass biochar had a CEC of over 60 cmol/kg whereas high temperature oak biochar has significantly lower CEC of around 10 cmol/kg. This supports the findings of the same group that increased pyrolysis temperature results in a

loss of polar functional groups. In over-simplified trends, low temperature pyrolysis increases the CEC of the resulting biochar, however, it also lowers the aromaticity, the surface area and pH of the biochar, which are favorable qualities for a soil amendment to have. Yet even with a lower CEC, the oak biochar may have effects that are beneficial to severely degraded soils. Terra Preta soils have been found with an average CEC of 10-15 cmol/kg, while neighboring Oxisols have CEC values of just 1-2 cmol/kg (Glaser et al., 2001). Addition of the aforementioned oak biochar would be beneficial to such a soil, however to maximize the characteristics that are potentially beneficial to less degraded soils, such as the local CT river basin region. To determine a suitable biochar amendment for a specific soil in question, the soil must be analyzed completely before a potentially beneficial biochar product can be identified. There is no universal biochar product that can benefit every soil type.

2.3.4 Surface Area

Wide-ranging characteristics were found among the resulting biochars and it is the specific characteristics of each biochar have a large affect on its potential benefit to soil fertility. For instance, the amount of surface area that a biochar has can directly affect its cation exchange capacity as cation exchange occurs on the surface. The highest amount of surface area observed in one study was obtained from pecan shell biochar produced at 700°C with a surface area of 222 m^2/g. The lowest surface area achieved was 0.40m^2/g which was from switchgrass biochar produced at 250°C (Novak et al., 2009b). The immense difference was due to both the biomass and the temperature of pyrolysis. No two biochars are alike. What this work highlighted, though, was that there was a clear trend in surface area based on pyrolysis temperature. To generalize, as the pyrolysis temperature increases,

the resulting biochar formed from the same initial biomass will have increased surface areas. This indicates there is potential here to design biochar with specific characteristics that will have the desired effects on the soil in question. Produce the biochar at higher temperatures to increase the surface area for moisture retention or produce it at a lower pyrolysis temperature for biochar with increased in CEC to increase nutrient retention in soils (Novak et al., 2009a).

2.3.5 pH

A similar trend between temperature and pH is present in the pH of the biochars produced (Novak et al., 2009b). As production temperatures increased, the pH also increases. This is likely due to the increased concentration of inorganic elements in the higher temperature biochars as more organic material was combusted and released as gas or bio-oil. An additional significant finding from Novak's study was that both the negative surface-charge density and number of acidic functional groups on biochars produced from the same feedstock, but at higher temperatures, decreases. This will greatly affect the cation exchange capacity of the biochar produced, which may have the greatest potential for positive soil amelioration (Novak et al., 2009b). If this work can be boiled down to a specific contribution to the study of biochar, it is that with the correct biomass as feedstock and the correct temperature of pyrolysis, a biochar may be produces with the specific qualities needed to ameliorate a specific soil, i.e. Designer Biochar.

2.4 Biochar Soil Interactions

2.4.1 Cationic nutrient retention

Nutrient leaching is a major issue in agricultural soils. There can be a large loss in soil fertility; a reduction in crop yield, increased soil acidification and on top of those issues, the lost nutrients can contaminate both ground and surface waters (Laird et al., 2010). However, significant improvements in soil fertility have been reported with application of biochar at levels as low as 2% by weight (Novak et al., 2009a). This can be attributed to the potential for increased SOM and CEC. Increased CEC enhances soil ability to hold and exchange positive plant nutrients such as ammonium, calcium and potassium. As was just presented, the CEC of biochars can vary significantly. It is likely though, that the CEC of a soil will increase with the added amendment of a high CEC biochar as soils CEC values are typically less than 20 cmol/kg (Mukherjee at al., 2011). Other soil macronutrients that are negatively charged, such as nitrate has reportedly shown reduced leaching in biochar-amended soils (Knowles, 2011; Ventura, 2012; Glaser et al., 2002). This would not be through cation exchange as these are negatively charged and would normally repel from the like-charged biochar surface. The mechanism for increased nitrate retention is unclear, but several theories have been proposed by Ventura (2012) including: microbial immobilization of nitrate, increased N uptake by plants, decreased water percolation, inhibition of nitrification.

2.4.2 Biochar and Soil Nitrogen

Other nitrogen interactions with biochar are less understood. There is a noticeable lack of understanding on how biochar interacts with nitrate-N in the soil (Steiner et al.,

2008). Studies have shown biochar to adsorb nitrate at high concentrations and remove it from solution (Mizuta et al. 2004). Potentially, ammonium adsorption onto the biochar surface could retard the leaching of nitrate through slower rates of nitrification (Clough and Condron, 2010; Taghizadeh-Toosi et al., 2011), yet the exposure of ammonia to biochar seemed to cause a disappearance of nitrate in biochar-amended soils (Spokas et al., 2011). Moisture content of the soil may also affect the nitrate concentration, since drier soils tend to have slower nitrification rates than those at field capacity.

There are many conflicting findings on an integral aspect of the soil nitrogen-biochar relationship (Steiner et al., 2008; Singh et al., 2010; Knowles et al., 2011; Yao et al., 2012; Clough et al., 2013; Hale et al., 2013). Recent studies point to the changes in the soil's physical properties as the main benefit of biochar in nitrate retention in the form of lower hydraulic conductivity (Kameyama et al., 2012) or greater denitrification rates may have been stimulated by increased carbon content of the soil (Schomberg et al., 2012). Specific adsorption of nitrate to biochar surface groups is also presented as the cause of nitrate retention in biochar amended soils (Dempster et al., 2012; Yao et al., 2012). If direct adsorption of nitrate is not occurring, then there is still the possibility for divalent or trivalent cations to act as cation-bridges between the negative biochar surface groups and the nitrate anion (Mukherjee et al., 2011). These wide-ranging theories of increased nitrate retention and/or lowered nitrate leaching invites more research on the phenomena. Especially as research has shown that effects on nitrate retention have occurred long after the initial biochar application, suggesting that the biochar surface does indeed change over time either through microbial oxidation or other factors (Singh et al., 2010).

Significant research efforts should be placed on better understanding the interactions between biochar and nitrogen forms in the soils. There is a consensus, though,

that this is an immensely important area of study. "Understanding a priori the interaction of

black carbon materials on nitrogen is desirable, otherwise, it may have properties that

could infringe on soil nitrogen availability and inadvertently reduce crop yields" (Spokas et

al., 2011a). Until the interaction of biochar with nitrogen cycling in the soil is better

understood, the widespread use of biochar as a soil amendment is hindered.

2.4.3 Biochar and Soil Phosphorous

Similar to the lack of understanding of the nitrogen biochar relationship in the soil,

phosphorous and biochar interactions are also under investigation. However in contrast to

the microbially-dominated nitrate dynamics in the soil, soil phosphate availability also

depends greatly on other soil chemical properties (DeLuca et al., 2009). Phosphorous, in

phosphate form, is often found as an available and extractable component of biochars made

from woody tissue such as pine and fir (Gundale and DeLuca, 2006). However, once in the

soil, alkaline biochars with mostly cationic exchange sites cannot attract phosphate

electrostatically. However, it was proposed by Lehmann et al., (2003) that increased

retention of phosphate may occur through complexation or cationic bridging with di- and

tri-valent cations.

2.4.4 Moisture retention

The potential shift in nutrient retention can affect several soil factors that in turn

influence the overall soil quality. The water holding capacity (WHC) of a well-aggregated

healthy soil is often greater than that of a degraded soil with little organic matter. Through

the addition of biochar, the WHC can increase significantly, through both improved soil

structure and increased porosity (Downey et al. ,2009). The significant porosity of biochar,

allows for water retention during dry periods, which can potentially alleviate water stress,

and simultaneously keep the soil well aerated. The pores of biochar range in diameter from

nanometers to micrometers and can hold a significant volume of water. The biochar

particles themselves allow for greater macropore space within the soil structure, which

allows for better drainage while also holding more moisture. The implications of biochar for

agriculture in arid climates are great, as water holding capacity has been reported to

increase as much as 18% in biochar-amended soils (Glaser et al., 2002). Again, this may

potentially impact the irrigation needs of agricultural soils and crops.

The increased water holding capacity also impacts the severity of leaching and

leaching events from agricultural fields. Nitrate is main source of nitrogen for plants and it

is highly mobile in the soil due to high solubility in water. Nitrate applied as fertilizer is

quickly leached downwards through the soil column with irrigation and precipitation or is

lost as surface run-off. With increased water holding capacity and soil porosity, more water

and therefore more dissolved nitrate, can be retained in the soil and kept available to plants

and microbial communities (Kameyama et al., 2012; Glaser et al., 2002). This is beneficial

both to the overall growth and health of the crop as well as the financial sustainability of

crop production. With higher retention of nitrate in the soil, plants will require less nitrogen

fertilizer during the growing season.

2.5 Biochar and Soil Biota

2.5.1 Nematodes and the Soil Environment

Complementary to microbial community analysis, nematode community structure can also be a viable bio-indicator of environmental health and useful as they are generally easy to sample, they are relatively stable communities in the soil and are the most numerous animal on the planet (Ferris and Ferris 1974; Bongers 1990; Visser and Parkinson; 1992; Bongers and Bongers 1998; Bongers and Ferris, 1999; Neher, 2001). Yet, nematode response to biochar amendment has been largely overlooked and under-reported in literature (Lehmann et al., 2011; Ameloot et al., 2013). Nematodes vary in their responses to pollutants and disturbances in their environment as the analysis of these responses in community structure can be a powerful tool for in situ assessment of soil health.

Nematode identification is helpful for biological assessment of soils for many reasons including these expressed by Bongers and Bongers (1998): (a) nematodes occur everywhere where decomposition takes place, (b) their morphology reflects feeding behavior, (c) of their interactions with other soil biota, (d) of their food-specificity, (e) they have a short response time, (f) they are easily isolated from the substrate and (g) genus identification is relatively simple. Identification of nematode morphological features, including mouthparts, allows for identification of primary food sources for each identified genus as well. Primary feeding types of nematodes include plant feeders also called plant parasitic (PP), fungal feeders (F), bacterial feeders (B), predatory feeders (P), and omnivorous feeding (O) (Yeates et al., 1993). While identification and key community indices are relatively easy to assess, characterizing the ecological shifts and relationships of nematodes in a soil environment is far less so.

In order to more easily use nematodes as environmental indicators, they must be identified and organized into their feeding groups (Yeates, 1984) as well as assigned colonizer-persister (CP) values, which represent their reproductive strategies (Bongers,

21

1990; Bongers and Bongers, 1998; Ferris et al., 2001; Yeates et al., 1993). Colonizers

reproduce quickly and in large numbers when the environmental conditions are healthy

and undisturbed, while maintaining resistance to environmental disturbance. In contrast,

persister families of nematodes reproduce in much smaller numbers and are rarely the

dominant species in any given ecosystem. Persisters are also more susceptible to ecological

disturbance (Bongers, 1990). The CP value ranges from 1-5, which 1 representing extreme

colonizer behavior and 5 representing extreme persister behavior. The use of feeding

groups and CP values is vital in assessing soil ecosystems and food webs. It is so far

impossible to determine the effect that a single species of nematode has on its environment,

therefore, larger groupings and assessment scales are necessary for ecosystem evaluation

(Bongers and Bongers, 1998). Although, species can be individually identified using

identification keys which have become much more comprehensive.

Several indices have been developed to try and characterize the population of

nematodes within a sample ecosystem, often referred to as nematode assemblages

(Bongers, 1990). The Shannon diversity index (Shannon, 1948) calculates the diversity

within a sample and allows for between sample comparisons using the following equation:

$$H' = \sum p_i \ln p_i$$

where p_i is the proportion of individuals in the (i)th family. The maturity index (MI)

developed by Dr. Tom Bongers is useful when comparing samples before and after

environmental changes or disturbances, or across a treatment gradient. It is less useful

when analyzing difference soil types and environments for simple comparisons. Lower MI

values indicate greater concentration of colonizers, which are more resistant to stress and

disturbance to the ecosystem. Higher MI values indicate more stable communities with greater numbers of persister families. The maturity index is calculated as follows:

$$MI = \sum_{i=1}^{n} v(i) * f(i)$$

where v(i) is the cp value of the identified family, and f(i) is the frequency of that in the sample (Bongers, 1990). This index is calculated using only non-plant parasitic nematodes. A plant parasitic MI can also be calculated in the same manner but is referred to as PPI.

While the maturity index provides a simplified tool to compare nematode communities in related soils, or experimental treatments, it does not offer detail into the structure of the community. Additional nematode community indices (Enrichment, Community and Structural Indices) have been developed to evaluate the differences among communities while trying to account for changes in specific taxa that indicate the abundance of sub-groups of nematodes, called functional nematode guilds (Ferris et al., 2001).

Biochar and its impacts on the soil nematode population have been largely under-reported in the scientific literature. In the most recent studies that have been reported, only the ratio of free-living nematodes to plant parasitic nematodes as affected by poultry-litter biochar has been evaluated (Rahman et al., 2014). It was reported by Rahman et al., that the addition of biochar to a vineyard soil in New South Wales, Australia lowered the plant-parasitic nematode population the greatest. This work was the second such study to report the reduction of plant parasitic nematode populations in biochar amended field soils. In an earlier study, biochar produced from wheat straw was shown to decrease the overall plant parasitic nematode abundance when applied at levels of 12 and 48 t ha^{-1} as well as increase

the fungivore population with no significant effect on overall nematode abundance across biochar application rates (Zhang et al., 2013). These are the only two studies to date that take a closer look at the effects of biochar on the nematode community in field trials. In a reported pot trail, an increase in one species bacterial-feeding nematodes (*Acrobeloides* sp.) was reported when soils were amended with 1 and 2% grass biochar (Fox et al., 2014). This particular bacteria-feeding nematode has a CP value of 1, indicating that it is a fast reproducer and likely able to increase population due to an increase in overall bacteria population in the biochar amended pots.

2.5.2 Biochar and the Soil Bacterial Community

Soil microbial communities are complex and the interaction of these communities with nutrient cycling is even more so. There are many taxa involved with the decomposition of organic material including bacteria, fungi, nematodes and more. The addition of biochar to soils can affect these microbial communities and their role(s) in the soil significantly (Warnock et al., 2007; Zimmerman et al., 2011). Many theories have been discussed and presented as possible explanations for the changes to the soil microbial community. For example, the same varied pore space that is found in biochars and increases the WHC can also affect the biota within the soil. One theory for increased microbial activity in biochar-amended soils is that the pore spaces give bacteria and fungal hyphae a physical refuge from predation (Warnock et al., 2007). Protozoa and nematodes that feed on these organisms are much larger in size and cannot physically enter the pore space within biochar particles. The average sizes of soil bacteria and fungal hyphae range from 1 to 4 μm and 2 to 64 μm, respectively, while a typical protist could be 100 μm. Simply analyzing the size differences and comparing them to a typical biochar pore space indicates that this is a

possible mechanism for increased microorganism diversity and activity in biochar-amended soils (Glaser, 2007; Warnock et al., 2007). The pores of biochar can also serve as a habitat for fungal hyphae (Thies and Rillig, 2009) and can then sporulate within the micropores leading to greater rates of mycorrhizae infection (Lehmann and Rondon, 2005).

Another theory regarding changes to microbial communities within biochar amended soils is that biochar has may ameliorate the presence of specific compounds, such as volatile organic compounds (VOCs) that can both inhibit and enhance microbial processes such as mineralization and reproduction (Graber et al., 2010; Spokas et al.; 2011b). Elad et al (2010) also suggests that the shifts in microbial diversity and population are due to changes in the presence of biocidal agents in the biochar. These changes can be potentially harmful if they reduce the nitrifying communities or beneficial if the biocidal agent present (indirectly) improves plant stress resistance (Elad, 2010).

Or potentially, changes in microbial activity, within a biochar-amended soil, are the result of changes to the pH of the soil. If acidic, the soil pH can be shifted towards neutral with the addition of most biochars. A more neutral pH is conducive to increased bacterial growth. As microbial populations are most affected by the pH of their environment, biochar applications have the potential to significantly shift the make-up and the growth of the community (Atkinson et al., 2010).

Unfortunately, a large and healthy microbial community may temporarily lower the nutrient availability in a soil recently amended with biochar. Nitrogen immobilization is often observed in freshly amended soils due to the high C:N ratio of the added biochar (Lehmann, et al., 2003; Steiner et al., 2008). Bacteria will utilize all available nitrogen while trying to degrade high carbon compounds making nitrogen temporarily unavailable to plants for uptake (Warnock et al., 2007). This is a temporary effect and nitrogen levels often

rise again shortly thereafter. Over time, the microbes in the soil will begin to oxidize the surfaces of the biochar particle increasing the negative charge density and therefore continually increasing the CEC of the biochar-amended soil. However, although surface oxidation may occur, the biochar itself is resistant to mineralization by the soil microbes. Kuzyakov et al., (2014) for that after almost 4 years, less than one percent of the carbon (labeled with ^{14}C) from an initial biochar application was recovered in microbial biomass. Studies have also reported increases in mycorrhizal fungi in the presence of charcoal (Lehmann et al., 2006). The long-lasting changes in both physical and chemical properties of a soil amended with biochar are causing shifts in the microbial communities of these soils (Kolton et al., 2011).

2.5.3 Analysis of Bacterial Community Changes

When the environment in which these communities inhabit changes rapidly, microbes can respond in varying modes of survival with some successful and some unsuccessful (DeAngelis et al., 2010). Thus, rapid environmental changes may cause shifts in the microbial community composition. The shifts in microbial community are often assessed using molecular analysis of 16S rRNA, as culture-based identification vastly underestimates population diversity as culturable bacteria are a mere fraction of the total bacteria present (Leff et al., 1995; Griffiths et al., 2000). However, due to the co-extraction of humic acids and other inhibitory substances, the soil microbial community can be challenging to assess in this manner. Recent advances in DNA sequencing, such as Quantum insights into Microbial Ecology (QIIME) and high-throughput sequencing like the MiSeq (Illumina, San Diego, CA) , are revolutionizing the analysis of microbial ecology (Caporaso et al., 2010a; Caporaso et al., 2012). QIIME is open-source software that aids in interpretation

of raw sequencing data. It allows for within- and between- sample diversity analysis and can aid in building phylogenetic trees (Caporaso et al., 2010b). Basespace, a cloud based analytical site and Illumina's answer to QIIME, also delivers a free and user-friendly interface in which distinct applications are available for a wide range of analytical techniques. These apps are free to use and include 16S Metagenomics analysis, which can be used to determine microbial community changes at all taxonomic levels, based on the millions of base-pair reads using one of the Illumina sequencing platforms.

Sequencing of the 16S rRNA gene (within the DNA) for prokaryotes and 18S for eukaryotes has moved to the front of microbial identification due to the ubiquitous nature in bacteria (and archaea) and also due the highly conserved and variable regions of these genes. The genes for 16S rRNA region of microbial ribosomes have changed very little over time. Sequencing of these changes allow for bacteria phylogenetic and taxonomic identification. RNA itself can be used in microbial community studies. RNA is an important indicator in environmental samples of the active microbial community, as rRNA and mRNA are generally indicators of 'functionally active microbial populations' (Torsvik et al., 2002). The rRNA concentration can indicate the growth of that specific bacterium (Rastogi et al., 2004).

It is important to note that in order to identify microbes at the genera and potentially species levels, there must be access to a large database of complete nucleotide sequencing (i.e. GenBank and RDP Silva). Recognized taxa of bacteria are rapidly expanding with faster, deeper and less expensive sequencing options. While there is a very large database of 16S rRNA data, identification to the species level is poor. For example, using 16S rRNA sequencing, *Pseudomonas florescens*, which excretes a pigment that can chelate iron is not distinguishable from *P. jessenii*, a phosphate-solubilizing bacterium (Janda and Abbott,

27

2007). Small differences in the 16S rRNA sequence are difficult to identify and recently

diverged species may appear as though they are the same species. Or in the case of

Aeromonas veronii, this bacterium has heterogeneity (difference up to 1.5%) within its own

16S rRNA, making it difficult to identify and catalogue (Janda and Abbott, 2007). Dunbar et

al. (2002) reported using models, that to document just 50% of the species in a soil sample

would need a sample size of 16,000 – 50,000 individuals. Also, after analyzing clone

libraries, Dunbar et al. found that over 50% of the total clones in the each of the four

libraries came from just a few bacterial divisions, namely acidobacterium and

proteobacteria.

There has been significant work towards building a set of soil bacterial and fungal

primers for PCR quantification (qPCR). Quantitative PCR gives real-time quantitative

analysis of the presence of bacterial and fungal groups through fluorescent output of a

marker molecule. As amplification occurs, that fluorescent signal is also amplified and the

presence of certain bacteria is detected. This is particularly applicable to soil microbial

ecology, due to the high speed and input of qPCR. If the primers used are narrowed down to

be more specific to your sample content then increase information can be retrieved. Fierer

et al. (2005) used a set of primers that works specifically with the soil microorganisms they

were interested in identifying. Using targeted primers, the relative abundance of taxonomic

groups within different soil samples can be assessed. Just as DNA extraction can inherently

bias data towards certain groups, the same can occur with qPCR. The relative abundance

between soil samples is valuable data, however the abundance within a soil sample may not

be an accurate value. There are many soil bacterial groups or members within a group that

are not targeted with qPCR primers (Janda and Abbott, 2007).

Together, identifying the physical changes and the nematode and bacterial community changes that result from the addition of biochar to agricultural soils can be a powerful analysis of soil quality.

2.6 Hypothesis

This study investigates the suitability of a readily available charcoal waste product for use as a biochar soil amendment is temperate agricultural soils in Massachusetts. Massachusetts has a vibrant agricultural community, especially along the fertile banks of the Connecticut River. The banks of the river are home to prime agricultural lands due to their development over time through lake bottom sedimentation and as flood plain deposits. To maintain the health and quality of these lands, farmers must engage in sustainable practices that maintain and even enhance their soil health and quality, while also protecting the Connecticut River. Our hypothesis was using a Missouri-kiln produced hardwood charcoal waste product (biochar) as a soil amendment in this region could be one potential strategy for improving physical, chemical and biological characteristics of soils, thus enhance overall soil health, reducing fertilization and irrigation, and maintaining profitability of field crops and specialty crops such as sweet corn. Based on these conditions and the review of literature offered above, several hypotheses are proposed:

(1) A kiln-produced hardwood charcoal waste product falls within the acceptable range of characteristics presented in the literature necessary to be categorized as biochar.
(2) Addition of this biochar to a temperate agricultural soil in Massachusetts with impact the soil health and quality: chemically, physically and biologically.

Specifically, the addition of an alkaline biochar amendment with significant ash content will alter the soil density, soil moisture, soil pH and nutrient content, and thus will impact crop yield the soil microbial community.

2.7 Objectives

The objectives of this study were to:

(1) Evaluate physiochemical characteristics of a hardwood lump charcoal waste by-product for use as a quality biochar soil amendment;

(2) Quantify and analyze nitrate retention and in biochar amended field top and sub-soils;

(3) Analyze select macro- and micronutrient retention in biochar amended soils;

(4) Examine pH shifts in biochar amended soils over time and across treatment levels;

(5) Quantify and analyze marketable yield of sweet corn in biochar amended field soils as compared to a control soil;

(6) Determine the nitrogen uptake and storage as nitrate in corn stalks as affected by biochar in the soil;

(7) Compare ear quality from sweet corn grown in biochar amended field soils as compared to soils with no biochar amendment;

(8) Observe free-living nematode community shifts in biochar amended soils and calculate community indices; and

(9) Analyze shifts in soil microbial community abundance and diversity using Next-Gen 16S rRNA sequencing methods.

Detailed information about experimental procedures to reach these objectives is discussed in the following chapters.

CHAPTER 3

PHYSIOCHEMICAL CHARACTERISTICS RESPONSE OF TEMPERATE AGICULTURAL SOILS TO APPLICATION OF HARDWOOD BIOCHAR

3.1 Introduction

The Connecticut Valley has fertile soils along the Connecticut River due to centuries of glacial lacustrine sediment build-up when the valley was covered by Glacial Lake Hitchcock. As the lake drained and lowered in depth, the river backs emerged, and over time additional alluvial flood sediment was deposited thus creating the prime agricultural soils present today. These are not the typical degraded soils investigated when looking at the use of biochar in agricultural soils, however, they are still in danger of over-use as agricultural practices intensify and small farms work to maintain viability in today's local food market. Also, the proximity of these agricultural lands to the Connecticut River increases the concern of excess fertilizer run-off and leaching. In this study, the effects of a potentially readily available biochar are investigated for its effects on the soil's physical and chemical characteristics most relevant to maintaining a high quality, healthy and productive agricultural soil.

Wide-ranging characteristics are found among biochars and it is the specific characteristics of each biochar have a large affect on its potential benefit to soil fertility. For instance, the amount of surface area that a biochar has can directly affect its cation exchange capacity as cation exchange occurs on the surface. The highest amount of surface area observed in one study was obtained from pecan shell biochar produced at 700°C with a surface area of 222 $m^2\ g^{-1}$. The lowest surface area achieved was 0.40m^2 $m^2\ g^{-1}$ which was from switchgrass biochar produced at 250°C (Novak et al., 2009b). The immense difference

was due to both the biomass and the temperature of pyrolysis. No two biochars are alike.

What this work highlighted, though, was that there was a clear trend in surface area based

on pyrolysis temperature. To generalize, as the pyrolysis temperature increases, the

resulting biochar formed from the same initial biomass will have increased surface areas.

This indicates there is potential here to design biochar with specific characteristics that will

have the desired effects on the soil in question. Produce the biochar at higher temperatures

to increase the surface area for moisture retention or produce it at a lower pyrolysis

temperature for biochar with increased in CEC to increase nutrient retention in soils (Novak

et al., 2009a).

Historically, elemental compositions have been used to describe various

characteristics of both coal and charcoal (Schimmelpfennig and Glaser, 2012). As pyrolysis

most often results in the loss of significant amounts of hydrogen, oxygen, and even methyl

groups (Amonette and Joseph, 2009), the resulting elemental composition tends to results

in higher percentages of aromatic carbon as structures condense and other elements are

lost during the heating process (Baldock and Smernik, 2002). There are various

spectroscopic methods available that can be used to approximate the aromatic carbon

percentage of the resulting carbon structure as mentioned in the previous section. However,

it may not be necessary to turn to spectroscopic methods to analyze the stability of the

resulting biochar. Van Krevelen diagrams have been used for decades to compare the

hydrogen to carbon ratio (H/C) and the oxygen to carbon ratio (O/C) in carbon materials

(van Krevelen, 1957). These elemental ratios can also be used to assess the degree of

carbonization in biochars, which correlates to the recalcitrance of biochar in soil

(Schimmelpfennig and Glaser, 2012).

A similar trend to that of surface area and production temperature occurs with pH (Novak et al., 2009b). As production temperatures increased, the pH also increases. This is likely due to the increased concentration of inorganic elements in the higher temperature biochars as more organic material was combusted and released as gas or bio-oil. An additional significant finding from Novak's study was that both the negative surface-charge density and number of acidic functional groups on biochars produced from the same feedstock, but at higher temperatures, decreases. This will greatly affect the cation exchange capacity of the biochar produced, which may have the greatest potential for positive soil amelioration (Novak et al., 2009b). If this work can be boiled down to a specific contribution to the study of biochar, it is that with the correct biomass as feedstock and the correct temperature of pyrolysis, a biochar may be produces with the specific qualities needed to ameliorate a specific soil, i.e. Designer Biochar.

Nutrient leaching is a major issue in agricultural soils. There can be a large loss in soil fertility; a reduction in crop yield, increased soil acidification and on top of those issues, the lost nutrients can contaminate both ground and surface waters (Laird et al., 2010). However, significant improvements in soil fertility have been reported with application of biochar at levels as low as 2% by weight (Novak et al., 2009a). This can be attributed to the potential for increased SOM and CEC. Increased CEC enhances soil ability to hold and exchange positive plant nutrients such as ammonium, calcium and potassium. As was just presented, the CEC of biochars can vary significantly. It is likely though, that the CEC of a soil will increase with the added amendment of a high CEC biochar as soils CEC values are typically less than 20 cmol kg^{-1} (Mukherjee at al., 2011). Other soil macronutrients that are negatively charged, such as nitrate has reportedly shown reduced leaching in biochar-amended soils (Knowles, 2011; Ventura, 2012; Glaser et al., 2002). This would not be

through cation exchange as these are negatively charged and would normally repel from the like-charged biochar surface. The mechanism for increased nitrate retention is unclear, but several theories have been proposed by Ventura (2012) including: microbial immobilization of nitrate, increased N uptake by plants, decreased water percolation, inhibition of nitrification.

Other nitrogen interactions with biochar are less understood. There is a noticeable lack of understanding on how biochar interacts with nitrate-N in the soil (Steiner et al., 2008). Studies have shown biochar to adsorb nitrate at high concentrations and remove it from solution (Mizuta et al. 2004). Potentially, ammonium adsorption onto the biochar surface could retard the leaching of nitrate through slower rates of nitrification (Clough and Condron, 2010; Taghizadeh-Toosi et al., 2011), yet the exposure of ammonia to biochar seemed to cause a disappearance of nitrate in biochar-amended soils (Spokas et al., 2011). Moisture content of the soil may also affect the nitrate concentration, since drier soils tend to have slower nitrification rates than those at field capacity.

There are many conflicting findings on an integral aspect of the soil nitrogen-biochar relationship (Steiner et al., 2008; Singh et al., 2010; Knowles et al., 2011; Yao et al., 2012; Clough et al., 2013; Hale et al., 2013). Recent studies point to the changes in the soil's physical properties as the main benefit of biochar in nitrate retention in the form of lower hydraulic conductivity (Kameyama et al., 2012) or greater denitrification rates may have been stimulated by increased carbon content of the soil (Schomberg et al., 2012). Specific adsorption of nitrate to biochar surface groups is also presented as the cause of nitrate retention in biochar amended soils (Dempster et al., 2012; Yao et al., 2012). If direct adsorption of nitrate is not occurring, then there is still the possibility for divalent or trivalent cations to act as cation-bridges between the negative biochar surface groups and

the nitrate anion (Mukherjee et al., 2011). These wide-ranging theories of increased nitrate retention and/or lowered nitrate leaching invite more research on the phenomena. Especially as research has shown that effects on nitrate retention have occurred long after the initial biochar application, suggesting that the biochar surface does indeed change over time either through microbial oxidation or other factors (Singh et al., 2010).

Significant research efforts should be placed on better understanding the interactions between biochar and nitrogen forms in the soils. There is a consensus, though, that this is an immensely important area of study. "Understanding a priori the interaction of black carbon materials on nitrogen is desirable, otherwise, it may have properties that could infringe on soil nitrogen availability and inadvertently reduce crop yields" (Spokas et al., 2011a). Until the interaction of biochar with nitrogen cycling in the soil is better understood, the widespread use of biochar as a soil amendment is hindered.

Similar to the lack of understanding of the nitrogen biochar relationship in the soil, phosphorous and biochar interactions are also under investigation. However in contrast the microbially-dominated nitrate dynamics in the soil, soil phosphate availability also depends greatly on other soil chemical properties (DeLuca et al., 2009). Phospherous, in phosphate form, is often found as an available and extractable component of biochars made from woody tissue such as pine and fir (Gundale and DeLuca, 2006). However, once in the soil, alkaline biochars with mostly cationic exchange sites cannot attract phosphate electrostatically. However, it was proposed by Lehmann et al., (2003) that increased retention of phosphate may occur through complexation or cationic bridging with di- and trivalent cations.

The potential shift in nutrient retention can affect several soil factors that in turn influence the overall soil quality. The water holding capacity (WHC) of a well-aggregated healthy soil is often greater than that of a degraded soil with little organic matter. Through the addition of biochar, the WHC can increase significantly, through both improved soil structure and increased porosity (Downey et al., 2009). The significant porosity of biochar, allows for water retention during dry periods, which can potentially alleviate water stress, and simultaneously keep the soil well aerated. The pores of biochar range in diameter from nanometers to micrometers and can hold a significant volume of water. The biochar particles themselves allow for greater macropore space within the soil structure, which allows for better drainage while also holding more moisture. The implications of biochar for agriculture in arid climates are great, as water holding capacity has been reported to increase as much as 18% in biochar-amended soils (Glaser et al., 2002). Again, this may potentially impact the irrigation needs of agricultural soils and crops.

The increased water holding capacity also impacts the severity of leaching and leaching events from agricultural fields. Nitrate is main source of nitrogen for plants and it is highly mobile in the soil due to high solubility in water. Nitrate applied as fertilizer is quickly leached downwards through the soil column with irrigation and precipitation or is lost as surface run-off. With increased water holding capacity and soil porosity, more water and therefore more dissolved nitrate, can be retained in the soil and kept available to plants and microbial communities (Kameyama et al., 2012; Glaser et al., 2002). This is beneficial both to the overall growth and health of the crop as well as the financial sustainability of crop production. With higher retention of nitrate in the soil, plants will require less nitrogen fertilizer during the growing season.

3.1.1 Field Site Characterization and History

A three-year field experiment was conducted at the University of Massachusetts Amherst Crops and Animal Research and Education Farm in South Deerfield, MA (42°28'37"N, 72°36'2"W). The soil in the research field is labeled as prime farmland and is a moderately well drained Winooski silt loam (coarse-silty, mixed superactive, mesic Fluvaquentic Dystrudept) with a high run-off class. The Kw value for this soil is 0.43, which is rather high in a scale from 0.02 to 0.69. The depth to water table is approximately 22-24 inches and mean annual precipitation at this site ranges from 37-51 inches (94-130mm). The mean annual temperature is 37 - 51° F (3 -10.5 ℃). The research site had previously been left fallow, but mowed consistently, for 2 seasons prior to use in this study.

3.2 Materials and Methods

3.2.1 Field Study Experimental Design

The research area consisted of twenty-five 3 meter x 6 meter plots with 1.5 meter buffers between neighboring plots. Initial field preparation included plowing and disking along with the application of 1 inch of compost (applied with manure spreader). The layout of the experiment was split-plot design with five replications. Main plots were allocated to five application rates of biochar; control-0%, 2%, 4%, 6%, and 8% by weight (equivalent to the rates of 0, 40.5, 81.1, 121.5, and 162.0 Mg/ha respectively) which was applied to the research plots by hand. Experimental plots were constant throughout the three years of study. Research plots were disked to incorporate the biochar into the top 15cm of soil by disc harrow. Sub-plots consisted of two nitrogen rates including 0 and 56 kg ha-1 in the

form of calcium ammonium nitrate (CAN), which was applied by hand at the V6-7 growth stage of corn in 2013 and 2014.

The biochar used in this study was derived from sugar maple wood and processed using a Missouri Kiln (maximum temperature of approximately 400° C) for use as hardwood lump charcoal. Biochar characterization is presented in the following section.

An early maturity sweet corn hybrid (*Zea mays L. cv Spring Treat)* was planted using a cone type distributor mounted on a double disc opening corn planter. An early variety of sweet corn, *Spring Treat*, purchased from Johnny's Selected Seeds (Winslow, ME) was used in this research study. *Spring Treat* is a short season hybrid variety of yellow sweet corn with cool soil vigor. Prior to planting each season, field plots were treated with glyphosate herbicide and soils was prepared with a disk harrow. Sweet corn was planted in three rows (0.75m spacing), centered in each plot at a rate of 60000 plants per hectare. A combination of *S*-metolachlor and atrazine (Bicep II Magnum, Syngenta Corp.) was used as pre-emergence herbicide. Corn plants were not irrigated at this location because rainfall is normally considered adequate.

3.2.2 Biochar Characterization

Biochar samples were thoroughly characterized for pH, available nitrate concentration, phosphate concentration, additional select nutrient concentration, and density alongside the following soil characterizations described below. In addition, samples of biochar were also sent off site for elemental analysis and surface area characterization. Elemental analysis was performed in duplicate using the MicroCube elemental analyzer (MicroCube, Elementar, Germany). Surface area, pore volume and pore width were

determined by both CO_2 and N_2 gas adsorption isotherm analyses, using BET, NLDFT and DR methods.

3.2.3 Soil Nitrate Concentration Determination

Soil samples were taken early in the growing season from the field plots in order to utilize the Magdoff pre-sidedress nitrogen testing protocol (Magdoff, 1991). Samples were also taken at the time of corn harvest. In 2012, three soil samples were taken from 0-20cm from each plot, combined and dried overnight. In years 2013, and 2014, three soil samples were taken from 0-20 and 20-40cm in each sub-plot, combined and dried overnight. From each master sample, 8.0 grams of soil was weighed out and shaken in 40mL of 1.0 mM $CaCl_2$ at 200 oscillations per minute for 15 minutes and then filtered using medium grade filter paper. Filtrate was then analyzed for nitrate by colorimetric determination using flow injection analysis (QuickChem 8000, by LaChat Instruments, Loveland, CO). All extracted samples were stored at 4^oC until processing. All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute, Cary, NC).

3.2.4 Soil Phosphate Concentration Determination

The post-harvest sample from each of the three years was also analyzed for phosphate concentration. Eight grams of soil was shaken in 40mL of Modified Morgan Extractant (McIntosh, 1969) at 200 oscillations per minute for 15 minutes, as recommended for our region by the North East Soil Testing laboratory Manual (Thomas Sims and Wolf, 1995). Samples were filtered using medium grade filter paper and diluted 1:5 with deionized H_2O. This was to lower the concentration of phosphate as well as lower the salt

concentration from the Modified Morgan solution as it interfered with colorimetric determination. Once diluted samples were analyzed using flow injection analysis (QuickChem 8000, by LaChat Instruments, Loveland, CO). All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

3.2.5 Soil Nutrient Concentration Determination.

Soil samples were analyzed for other nutrient analysis using microwave plasma atomic emission spectrophotometry (MP-AES). The same soil samples previously, extracted in Modified Morgan solution, were used. Filtrate will then be analyzed for K, Ca, Mg, Mn, Cu, Zn, Fe, and Al using the MP-AES Agilent 4100 (Agilent Technologies, Santa Clara, CA). All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

3.2.6 Soil pH Determination

Soil samples were analyzed for pH following the North East Soil Testing laboratory Manual (Thomas and Wolf, 1995). The pH meter was calibrated using a two-point calibration for a range of 4-10 on the pH scale. From each soil sample, 5.0g was weighed out and added to a small plastic beaker. Then 5 mL of deionized water was added and stirred vigorously for 15 seconds. Soil-water slurries were then left to sit for 30 seconds. Electrodes were kept in the slurry, being carful not to get just the overlaying supernatant and pH values were recorded. All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

3.2.7 Soil Gravimetric Moisture Content

Soil samples were collected in August 2013 and test for variations in the total moisture content. Samples were collected 2 days after a rain event. Crucibles were oven dried for 24 hours at 100°C and weighed before adding soil samples from three field replicates. Crucible weights were recorded with the added soil. Crucibles and soil were then again oven dried at 100°C for 72 hours and reweighed. Moisture content was then calculated as grams of moisture lost per dry soil weight. All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

3.3 Results and Discussion

3.3.1 Suitability of Biochar

This commercially produced charcoal by-product has characteristics that are consistent with other low-temperature charcoal stack, kiln-produced, and hardwood lab-produced and/or characterized biochars in the literature (Baldock and Smernick, 2002; Kuo et al., 2011). This is an important finding because this is technically a waste product of commercial hardwood lump charcoal production. Therefore the charcoal producer has no added cost of production and profits from the sale of a product previously seen as waste.

In typical Van Krevelen diagrams of the H/C and O/C ratios of biochars, the lower the value of both elemental ratios indicates greater dehydration of the carbon structure and loss of oxygen and hydrogen from the original biomass structure (Amonette and Joseph, 2009). The pyrolysis of *Pinus resinosa* sapwood by Baldock and Smernik (2002) at various

temperatures show a movement towards the origin for both the H/C and O/C ratios with increasing temperatures indicating increased dehydration. *Pinus resinosa* heated to 350°C gives H/C of approximately 0.5 and O/C of approximately 0.3. This aligns with the ratios determined in this study (H/C = 0.47 and O/C = 0.16) for the pyrolysis of *Acer saccharum* in a Missouri Kiln with typical production temperatures of 350-400°C. The lowered O/C ratio is likely due to fewer carboxyl (C=O) groups (resulting from increased dehydration) relative to C=C groups. This is likely a result of the lower Oxygen pyrolysis environment (Baldock and Smernik, 2002). The higher H/C ratio of the biochar used in this study indicates incomplete pyrolysis of the wood or incomplete demethylization (Schimmelpfennig and Glaser, 2012), leaving a more heterogeneous product with a higher hydrogen content and slightly lower aromatization. As Keiluweit et al. (2010) discuss, there is significant decomposition of cellulose and hemi-cellulose at temperatures under 400°C, however significant lignin decomposition requires higher temperatures. The char used in this study, can be assumed to have higher amounts of lignin subject to incomplete decomposition, resulting in the higher H/C ratio. Even with the higher H/C ratio, this biochar is still suitable and even desirable as a soil amendment and for carbon sequestration. The biochar used here falls within the threshold of sufficient amounts of aromatic carbon and relatively low numbers of functional groups proposed by Schimmelpfennig and Glaser (2012) of an H/C ratio of ≤0.6 and an O/C ratio of ≤0.4. The biochar used in this study also falls between fresh and oxidized biochars regions when plotted with a range of oxidized and fresh char as well as slightly charred biochar (Hammes et al., 2006).

The carbon content, as well as the type of carbon within a biochar can have a great effect on its recalcitrance in the soil. Highly condensed aromatic carbon biochars with little hydrogen and oxygen content tend to have greater resistance to microbial degradation and

thus last significantly longer in the soil (Harvey et al., 2012). The recalcitrance index (R_{50}) developed by Harvey et al. (2012), investigates the rate of oxidation of the biochar material in comparison to graphite, which is the most recalcitrant of carbon materials. Several biochars made from hardwood and at temperatures between 300°C and 400°C were included in this study of recalcitrance. The R_{50} of these biochars range from 0.46 to 0.51 and were classified as Class B carbons. Harvey et al. (2012) then used one-year incubation dates from Zimmerman et al., (2010) to try and draw conclusions between the R_{50} of diverse biochars and their rate of mineralization when incubated with soil at a rate of 10% biochar. Biochars with R_{50} values of approximately 0.5 lost less than 1% of their carbon content over the course of one year and later may induce a negative priming effect due to organic matter from the soil sorption onto the biochar surface (Zimmerman et al., 2011). These biochars are characteristically similar to the one used in this study, thus this data also supports the use of the hardwood charcoal production waste by-product as a legitimate biochar source.

Another consideration of the desirability of a biochar is its surface area. The Brunauer-Emmet-Teller (BET) surface area of the biochar used in this study is relatively low compared to other chars at an area of 17.9 m^2 g^{-1}. This is consistent with a wood char produced at a pyrolysis temperature between 300°C and 400°C (Keiluweit et al., 2010). Overall, this biochar, and other kiln produced chars should be given greater consideration for research and field use due to the greater availability as this technology has been long established (Brewer et al., 2011) and waste product from charcoal production falls within many of the recommended characteristics and parameters set forth for quality biochar in the literature.

3.3.2 pH

One of the most significant effects that a biochar may have is its influence on soil pH. Biochar pH is as wide ranging as the feedstock and pyrolysis methods used to produce it. More often than not, however, biochar has an alkaline pH and when added to soils, significantly affect the pH of those soils. Samples taken of the biochar used in this studies had a pH range of 8.0-8.3, which a mean pH of 8.1. The addition of an alkaline biochar to soil can have a liming effect and that effect is seen in this field study as well. Soil pH values rose from below a pH of 6 to 6.8 in the highest biochar treatment level. This increase in soil pH has persisted through the first three years of this long-term study and in fact the effect of the biochar on the pH has increased over time. In year 1 of this study, the average maximum pH affect measured was in the highest treatment level of 8% biochar with an increase of approximately 0.5. This initial effect can be attributed to both the alkaline pH of the biochar itself, but also to the release of a significant amount of the base cations, Ca^{2+}, Mg^{2+} and K^+ from the biochar. These base cations will displace acid cations such as H^+ and Al^{3+} from soil colloid surfaces, allowing the H^+ to leach away or be neutralized in the soil solution and the Al^{3+} to precipitate out as an oxide or hydroxide, thus decreasing both the active and reserve acidity of the soil. This is reflected in the lower available Aluminum concentration of biochar-amended plots (see Table 5).

However in both years 2 and 3 of this study, the differences observed between the control soil and the 8% treatment level increased reaching a difference of >0.8. This increase in the soil pH over time is likely due to continued removal of acid cations through displacement by base cations (Mg, Ca, and K) as well as increased microbial oxidation of the biochar surface, increasing the number of surface oxygen-containing functional groups and thus the pH. It is important to note, that all biochar treatment levels significantly increased

the soil pH. Even the unusually large amount of biochar applied at the highest treatment level (8% or 162 metric t ha^{-1}), maintained pH values within the acceptable range for field crop growth. The observed pH shifts in this research work agree with other published studies as well (Lehmann et al., 2003; Steiner et al., 2007; Vaccari et al., 2011). Discussion on the pH and nutrient availability continues in the nutrient specific discussions below.

3.3.3 Moisture Content

Biochar, as a porous material, has also shown to affect surface water infiltration, water-holding capacity and thus mediate drought stress (Downie et al., 2009; Karhu et al., 2011). The biochar used in this study was investigated for changes in the gravimetric moisture content 48 hours after a rain event in the summer of 2013. Precipitation in the amount of approximately 50 mm was received on August 4th, 2013 followed by two full days with zero precipitation and high temperatures of 75° and 77°F on August 5th and 6th, respectively. Soil samples were taken on August 7th and immediately weighed and dried to determine gravimetric moisture content (GMC).

While no significant differences were found among the biochar treatment levels at the 20-40cm depth, the GMC increased at the 0-20cm depth. This is consistent with the addition and incorporation of biochar from 0-15 cm. The increase from approximately 23.0% GMC to 24.4% in the 6% biochar treatment level can be attributed to the biochar retaining water in its porous structure. At the 2% biochar treatment level, no increase was seen as compared to the control soils. A non-significant increase was seen at 4, 6, and 8% biochar treatment levels, with 6% having the greatest GMC. The decrease at the 8% treatment level may be due to an increase in surface evaporation as significant amounts of

biochar accumulate on the surface after rain at the 8% treatment level. This would suggest a

higher recommended application rate of biochar in arid regions with little precipitation to

improve soil moisture retention and thus lower irrigation rates. The annual precipitation at

the field site is sufficient for most crops without additional irrigation. Therefore assessing

the influence of biochar on mitigating drought stress is not applicable to this study.

3.3.4 Phosphate

The overall nutrient content of the biochar used in this study is high (Table 4).

Available N is low, as nitrogen is the most sensitive to heating, and likely to be low in many

biochars, especially those made form a plant-based biomass (manure biochars tend to have

higher available N content) (Krull et al., 2009). The extractable phosphate (using *Modified*

Morgans Extracting Solution) content of the biochar in this study ranged from 98 to 142 mg

kg^{-1} with a mean of 115mg kg^{-1} and this value was confirmed with secondary testing by the

UMass Soil Testing Laboratory (using Morgans Extracting solution). There is documented

release of P from char in the research literature from over 60 years ago (Tyron, 1948).

Woody tissues release phosphate salts during the charring process, as P volatilization is not

affected until temperatures of 700°C or higher are reached (DeLuca et al., 2009). The char in

this study, produced at approximately 350-400°C has a high available P concentration. This

is due to the cleaving of organo-phosphate bonds as the carbon within the plant tissue is

volatilized or structurally altered. This phosphate has been well documented as available

and being released to amended soils in the literature (Lehmann et al., 2003; Yao et al., 2012;

Hale et al., 2013; Hollister et al., 2013). Phosphate availability is also affected by the soil pH.

The soils in the study showed an increase in pH from approximately 5.8 in the control soils

to over 6.8 in the highest treatment level. This full point increase in pH has a significant

influence on the availability of phosphate in the soil, with the greatest positive affect occurring when a pH of 6.5 or greater is reached. All biochar-amended soils have pH values that fall within the range of soil pH that corresponds with the most availability of the present phosphorous in soil. Alkaline soils (pH>8) cause increased precipitation of phosphorous with calcium, and acidic soils (pH<6) increase the precipitation of phosphorous with aluminum and precipitates even more with iron when the soil pH is below 5 (Marschner, 1995). The retention of the phosphate anion has been attributed to increased coordination of phosphate to biochar-sorbed calcium. This phosphate has been reported to maintain its availability to plants, unlike precipitated calcium phosphate in unamended soils (Major et al., 2009). The combination of the liming effect that the biochar has on the acid soils of this study and the release of phosphate from the biochar itself result in greater available phosphate in all levels of biochar treatment.

3.3.5 Base Cations: Calcium, Magnesium and Potassium

The concentration of base cations in soils can affect both soil physiochemical characteristics as well as plant growth. The introduction of base cations, Mg, Ca, and K, into the soil can aid in increasing the pH through both active and reserve acidity displacement. These cations will replace acid cations, such as Al and H, in cationic exchange sites allowing those acid cations to be neutralized, leached away or precipitate out in unavailable forms such as aluminum hydroxide. With the addition of significant amounts of these base cations, the soil can also increase its percent base saturation, which can buffer future pH changes as well. The biochar used in this study consisted of over 18% mineral ash. This mineral fraction can have a significant effect on soil properties as the biochar weathers and releases these minerals to the surrounding soil solution (Yao et al., 2010).

The biochar used in this study contains 869, 10771, and 3578 ppm of magnesium, calcium, and potassium respectively. At a biochar application rate of 2% (w/w) this introduces over 17 ppm of magnesium, 215 ppm of calcium, and 71ppm of Potassium to the amended soil. This is reflected in the significant increase in these cations in the biochar-amended soil. In the first year of this study, the available Ca, Mg, and K in the soil increased, but only potassium increased significantly. The control soil contained 179.5 ppm K, whereas the highest biochar treatment of 8% had 354.9ppm of available K. The soil samples for this analysis were taken in August of 2012, less than 2 months after biochar application. The calcium and magnesium contained within the biochar pores may remained sorbed to the biochar surface, not yet releasing into the soil solution and thus was limiting the availability and extractability within the set procedure.

By the following August in 2013, all three base cation concentrations were significantly larger in biochar amended soils. Magnesium ranged from 134.8ppm in the control soil to 205.0ppm in the highest treatment level. Calcium ranged from 1523 to 2515 ppm and potassium increased from 153.5 to 424.1ppm. These increases continued and similar significant increases were present in the August 2014 samples as well. This almost immediate increase in potassium and delayed increased in calcium and magnesium was also seen with the addition of both maple and spruce biochar to forest soils (Sackett et al., 2014). The fast release of K from biochar is likely due to its high solubility and the delayed Mg and Ca from biochar can be attributed to increased charge compared to K, but also to lower solubility and potential for precipitation with anions such as carbonates (Novak et al., 2009b; Yao et al., 2010). Overall the increased concentration of all three base cations in biochar-amended soil was significant, and concentrations remained elevated indicating sustained retention of these plant nutrients in the biochar treatments.

3.3.5 Aluminum and Iron

Highly acidic soils also tend to be high in aluminum and iron concentrations. Aluminum is of particular importance to agriculture, as the Al^{3+} ion causes lysis of water molecules, releasing hydrogen ions into the soil solution. The released hydrogen ions displace soil nutrient cations from soil colloid surfaces which are subsequently leached away, thus decreasing nutrient availability to plant roots. This exacerbates the acidic conditions, spurring more soluble aluminum and hydrolysis, creating aluminum phytotoxicity. To alleviate aluminum toxicity, farmers often lime the soil to increase the pH. Increased pH causes aluminum to form aluminum hydroxide species, which are less soluble in the soil solution as well as less phytotoxic (Marschner, 1995).

As alkaline biochar amendments have shown to increase soil pH thus a similar liming effect is often observed (Lehmann et al., 2003; Steiner et al., 2007; DeLuca et al., 2009; Vaccari et al., 2011). An associated reduction of soluble aluminum has also been reported when biochar raised soil pH after application (Steiner et al., 2007). The control soil used in this study maintained pH values below 6, where as the biochar amended soils all had elevated soil pH, increasing over the three year study and maintaining pH values of 6.2-6.8 (lowest biochar-highest biochar treatment, respectively). This correlated to a decrease in available aluminum concentration of (Figure 9). This effect may be more sustainable than shorter lasting pH changes due to application of lime (Qian et al., 2013).

Iron responds to changes in the soil pH similarly to aluminum. As the pH of the soil increases alkaline iron solubility decreases. Alkaline soils are in danger of causing iron deficiency in plants due to the transformation of iron into unavailable oxide species. In this

50

work, available soil iron concentration decreased with the addition of biochar. The decreases seen amongst the control and biochar treatment levels were non-significant in the first two years. In 2014, the decrease in available iron was significant (*P>0.05*), noting a decline in available iron from 3.35ppm in the control soil to 2.18ppm in the highest biochar treatment level.

There are limited studies that report on the effect of biochar amendments and iron availability in soils. However, a recently published report by Alburquerque et al. (2015) investigated iron uptake by lupin and sorghum when soils were amended with varying biochars and iron fertilization in a pot experiment. Application of a pine biochar and an olive pruning biochar to iron deficient soils resulted in lower leaf iron content and no change in the overall soil iron concentration. The lower leaf content may be due to the increase in soil pH. Yet, when those deficient soils were fertilized with iron sulfate, both biochar amendments showed an increase in leaf iron content (Alburquerque et al., 2015). This study did not investigate the iron content of corn tissue, but the reduction in available iron in biochar amended soils agrees with the aforementioned study, that the pH shift towards neutral from acidic is causing the decrease in available iron in biochar amended soils.

3.3.6 Zinc, Manganese, and Copper

There were no significant changes in the concentration of available zinc in the biochar-amended soils assessed in this study. The biochar itself contained on average 13.35 ppm zinc, but saw no significant release of zinc from the biochar or immobilization of pre-existing soil zinc.

Available manganese in the biochar-amended soil showed a significant increase as the biochar application rate increased in the first year of this study, 2012. The biochar introduced on average 467.8 ppm Mn to the soil upon amendment. This accounts for the immediate increase in Mn availability. Overall, available manganese concentrations declined in the 2013 and 2014 growing seasons and in year 2014 no significant differences in available manganese were detected. This may be attributed to the increase in soil pH with biochar amended soils, as increased pH can cause manganese to precipitate leaving soils with a pH over 6.5 prone to manganese deficiency (Hodges, 2010).

The sugar maple biochar used in this study was not a major source of copper, with less that 1ppm of copper extracted from biochar samples. The effect of biochar amendment on the soils in this study was initially significant. In 2012, shortly after biochar application, available copper concentrations declined in the soil. This could be due to an increase in the soil pH, which decreases the solubility of copper minerals as reported by Beesley et al. (2010), but available copper concentrations were not significantly different in years 2013 and 2014, when the pH differences were greater.

3.3.7 Nitrate

The retention of nitrogen in agricultural soils is always of significant interest to farmers and agricultural researchers. Nitrogen is often a limiting factor in crop production, and even when it is applied to the soil, it is difficult to keep nitrogen from leaching as nitrate or volatilizing in the summer months as ammonia. Significant efforts have been made in agriculture to lower nitrogen applications and lower nitrogen leaching which can contaminate both ground and surface waters. The relationship between nitrogen in the soil

and biochar applications is of great interest, as biochar has been shown to increase soil's capacity for moisture retention (Karhu et al., 2011) through greater soil porosity and through the biochar's porous structure itself (Downie et al., 2009).

Studying interactions between nitrogen in the soil and charcoal is a logical next step to take. The potential for greater nitrogen retention, especially as nitrate seems logical as nitrate is highly soluble in water, and if charcoal or biochar can increase the moisture retention is soils, then more nitrate should be retained as well. However, there has been conflicting evidence presented in the literature regarding the retention of nitrate in biochar-amended soils. Select studies report the adsorption of nitrate to biochar surfaces (Mizuta et al., 2004; Knowles et al., 2011; Kameyama et al., 2012;); reduction of nitrate leaching in biochar amended soils and sans (Laird et al., 2010; Yao et al., 2012), while others show little or no sorption of nitrate at all (Yao et al., 2012; Hale et al., 2013). The greatest influence on a biochar's ability to retain nitrate is still up for great discussion, as the results presented in the literature range as greatly as the properties of the biochars used in those same studies.

Nitrogen availability was shown to decrease with the addition of 10% charcoal to both a Xanthic Ferrasol and a Fimic Anthrosol, with little effect on the overall plant biomass. Nitrogen (as ammonium) leaching was significantly reduced with the addition of charcoal, however nitrate leaching was slightly higher in the charcoal amended treatments (Lehmann et al., 2003b). This overall retention, yet unavailability of nitrogen in charcoal amended treatments was attributed to nitrogen immobilization due to the high C:N ratios of the charcoal treated soils (Rondon et al., 2007) . Corncob and cacoa shell biochars produced at a similar temperature to the biochar used in this study saw no significant sorption of nitrate or release of nitrate from biochar (Hale et al., 2013).

Significantly lower available nitrate-nitrogen was observed in the biochar-amended plots two weeks after application at the 0-20cm depth (Figure 9). Interestingly, nitrate levels in the biochar-treated plots surpassed the control by the end of the growing season (Figure 10) although there was no statistical significant difference amongst treatments. The same early season depression of nitrate availability was observed again in the second year of this study, yet with no significant difference among treatment levels at time of harvest. Not surprisingly, however, with the addition of 56kg ha^{-1} of calcium ammonium nitrate (CAN) at the V6-V7 growth stage of sweet corn, which is typical timing for the pre-sidedress nitrogen test (hereafter referred to as PSNT), greater nitrate concentration was observed as compared to the non-fertilized plots at the 0-20cm depth. There was also no significant interaction between fertilizer and biochar application. In year three of this study, early season immobilization at PSNT was limited to only the 6 and 8% biochar treatment levels at the 0-20cm depth. This can be partly attributed to the continued efforts of the microbial population to mineralize any remaining labile carbon compounds that remain in the highest application rates. The early season nitrate concentration in the 2 and 4% treatment levels showed no statistical difference as compared to the control at the 0-20cm depth.

Nitrate concentration was also analyzed at the 20-40cm depth in the second and third years of this field study. In both, years nitrate concentration was initially higher in the lower depth of soil at PSNT. As each season progressed, 20-40cm depth nitrate concentrations diminished at all biochar treatment levels. This indicates reduced leaching of nitrate downward through the soil column due to the addition of biochar. This lowered nitrate leaching supports increased nitrogen immobilization by the microbial community as there was also lower plant uptake of nitrogen (to be discussed in the following chapter) and lower available nitrate-nitrogen concentration in the biochar-amended soil at all. This

phenomenon was also reported by Guerena et al. (2013) in a NY maize-based cropping system. Small amounts of biochar may remain labile and mineralizable directly after application, thus stimulating bacterial and other microbial population growth and immobilizing nitrogen (Lehmann et al., 2010). Although this period tends to be short lived (Kuzyakov et al., 2009), with lower temperature biochars, such as the one used in this study, a greater percentage of the carbon may be mineralizable and the immobilization effect may last longer. Overall microbial immobilization plays a significant role in the retention of nitrogen in soil. Steiner et al. (2004) reported higher microbial populations with charcoal and glucose addition to soils, although they did not see a rise in overall respiration rates. This may be due to the inability of the microbial community to mineralize the stabile carbon structure of biochar and charcoal.

3.4 Conclusion

This study addressed the feasibility of using an often-discarded by-product of hardwood lump charcoal production as a suitable biochar for agricultural soil amendment. It was hypothesized that a kiln-produced hardwood charcoal waste product would fall within the acceptable range of characteristics presented in the literature necessary to be categorized as biochar. According to the characteristics of this particular waste-byproduct, as analyzed and presented in this study, it does fall within acceptable chemical characteristic values to be used as a soil amendment in agricultural soils. The pH, elemental composition, elemental ratios and potential recalcitrance all indicate that this product is suitable for increasing soil pH long-term in acid soils, increasing soil porosity and benefiting soil structure, increasing soil carbon content while maintaining resistance to soil microbial mineralization.

It was also hypothesized that the soil health and quality would be impacted by the addition of this biochar. Physical qualities were affected; soil density was lowered with the addition of biochar, and discreet increases in soil moisture content were observed (although not statistically significant). Chemical properties such as pH did significantly increase improving the growing condition of the soil by raising the pH into an optimal range for not only sweet corn but also many other agricultural crops. The overall soil CEC improved slightly with increased biochar application rates, however the percent base saturation was improved drastically due to the retention of important plant nutrients such as calcium, magnesium and potassium. Soil phosphate availability was increased likely due to the release of phosphate salts from the biochar itself and greater retention of phosphate due to cation bridging. This area of focus is still not fully understood and requires greater research into the interaction of biochar surface functional groups and phosphate retention. Soil nitrate availability was not significantly impacted, other than in an increase in immobilization due to the increase in soil carbon content which resulted in significantly lower available nitrate at PSNT in higher biochar treatment levels.
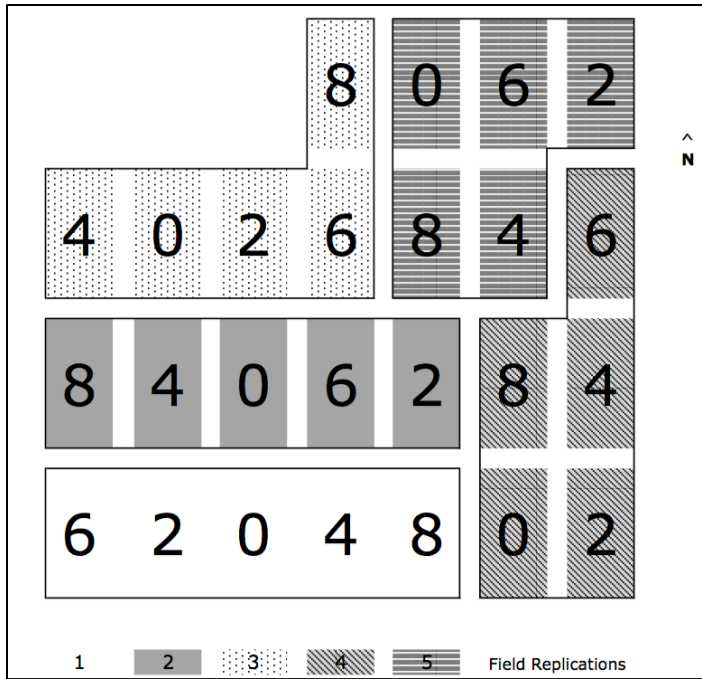
Figure 4: Plot layout of field study, University of Massachusetts Amherst Crops and Animal Research and Education Farm in South Deerfield, MA

Figure 5: Field Plots after compost and biochar application, but before incorporation with a disc harrow.

Table 1: Field Study biochar treatment application rates.

Biochar Treatment (%w/w)	Short tons acre[-1]	metric tons ha^{-1}
0%	0	0
2%	18.1	40.5
4%	36.2	81.0
6%	54.2	121.5
8%	72.3	162.0

Application rates were determined based on the top 6 inches of soil (incorporation depth), soil density and biochar density.

Table 2: Elemental analysis of sugar maple (*Acer Saccharum)* biochar.

Element	(%wt)
C	63.7
H	2.5
O	13.2
N	1.2
Ash	19.0

Atomic Ratios	
H:C	0.47
O:C	0.16
C:N	61.6
C:N by weight	52.8
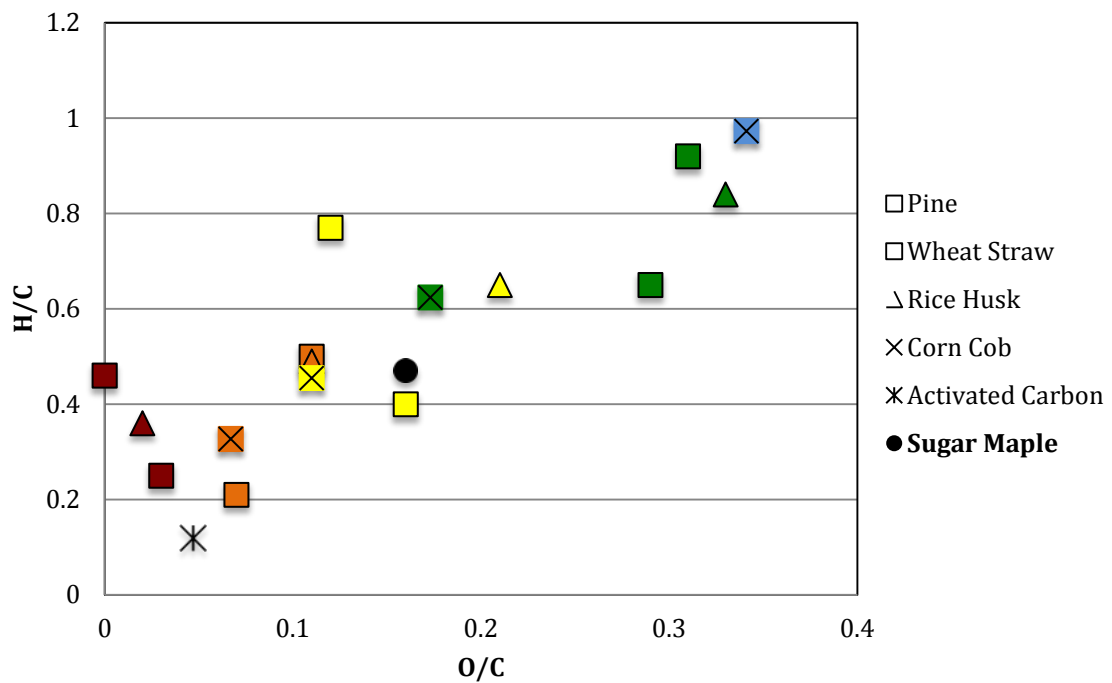
Figure 6: Van Krevelen Diagram of select published biochars and sugar maple (Acer saccharum) biochar used in this study.

(Zheng et al., 2010; Crombie et al., 2013).

Max temperature of production for sugar maple biochar is approximately 400°C.

Colors represent production temperatures.

Red = 650°C Orange=550°C Yellow=450°C Green=350°C Blue=250°C

Table 3: Sugar maple *(Acer saccharum)* biochar surface area and pore analysis.

	Multipoint BET Area*	17.90
Surface area	DR Method Micro Pore Area	76.21
($m^2\,g^{-1}$)	Mesopore Surface Area*	1.66
	NLDFT Method Cumulative Surface Area	63.03
	Total Pore Volume*	0.032
Pore volume	Micro Pore Volume*	0.011
($cc\,g^{-1}$)	NLDFT Method Cumulative Pore Volume	0.022
	DR Method Micro Pore Volume	0.027
	NLDFT/GCMC-Method Pore Width	57.30
	DR Method Micro Pore Width	116.00
Pore size (nm)	Average Pore Width*	71.09
	Micro Pore Width*	22.92

*Analysis using N_2 adsorption isotherms. All other analyses completed with CO_2.

Table 4: Extractable essential plant nutrients and aluminum from Sugar maple *(Acer saccharum)* biochar and initial soil nutrient analysis.

Element	Concentration, mg kg^{-1}	
	Biochar	**Field Soil**
P	115.2	11
K	3578	54
Ca	10771	710
Mg	869	50
Mn	468	2.0
Zn	13.3	0.5
Cu	0.92	2.0
Fe	16.8	5.0
Al	17	73
pH (1:1, H$_2$O)	8.1	5.6

Elements extracted using Modified Morgan solution as recommended by the Northeastern Soil Testing Handbook.

Samples ran in triplicate. Mean values presented in table.

Nutrient content determined by MP-AES unless otherwise noted.

*Colorimetric determination by flow-injection analysis.
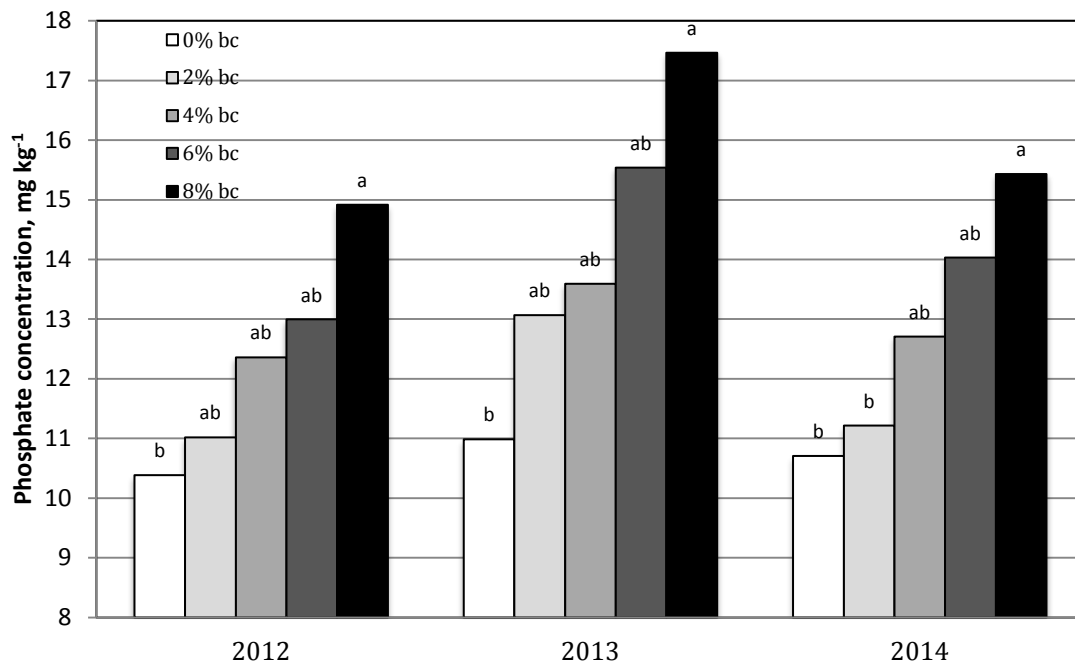
Figure 7: Annual soil phosphate assessment of biochar amended field plots.

Values presented are treatment means.

Means separated by sampling date.

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.
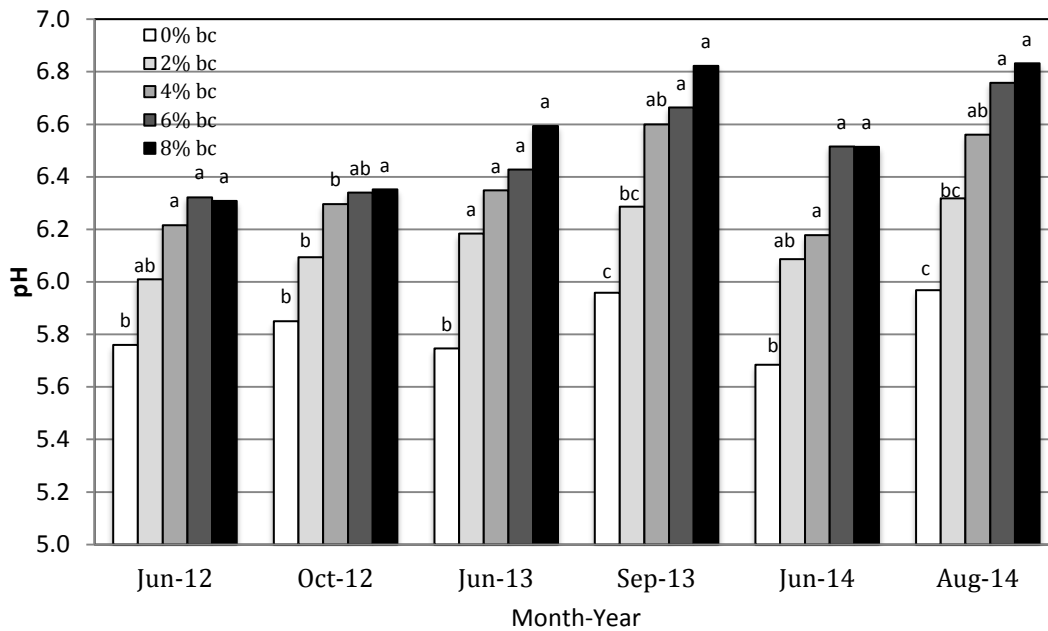
Figure 8: Pre-Sidedress and Post-harvest pH of biochar amended field plots.

Values presented are treatment means.

Means separated by sampling date.

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.
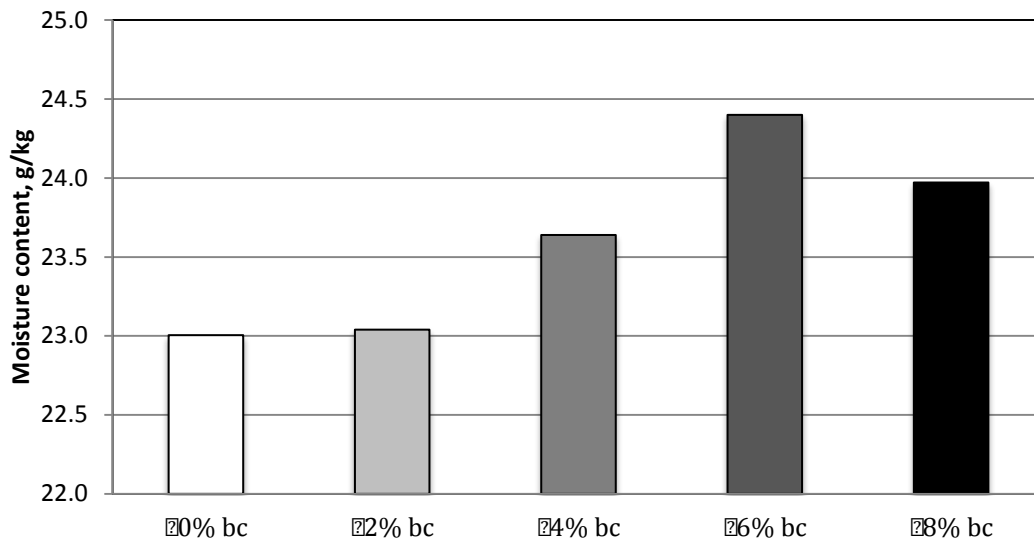
Figure 9: Gravimetric moisture content of biochar amended field plots.

Samples taken 48 hours after rain event.

Values presented are treatment means.

No significant difference found at *P< 0.05*.

Table 5: Select soil and biochar available ion concentration data

Year	Treatment	Nutrient Concentration (ppm)							
		Mg	Zn	Fe	Mn	Ca	Cu	Al	K
2012	0%	114.4[a]	2.16[a]	2.90[a]	15.37[b]	1316[a]	2.42[a]	80.19[a]	179.5[b]
	2%	128.2[a]	2.11[a]	2.45[a]	16.16[b]	1487[a]	2.05[ab]	72.30[ab]	233.0[ab]
	4%	140.1[a]	2.06[a]	1.97[a]	19.49[ab]	1581[a]	2.03[ab]	63.65[ab]	253.9[ab]
	6%	148.7[a]	1.80[a]	2.38[a]	20.45[ab]	1488[a]	1.67[b]	46.66[b]	243.9[ab]
	8%	157.9[a]	2.55[a]	2.37[a]	25.70[a]	1836[a]	1.97[ab]	61.14[ab]	354.9[a]
2013	0%	134.8[b]	1.97[a]	3.29[a]	12.56[ab]	1523[b]	2.19[a]	81.89[a]	153.5[c]
	2%	158.3[ab]	2.04[a]	2.37[a]	10.24[b]	1803[b]	1.93[a]	66.29[b]	262.9[bc]
	4%	182.3[ab]	1.98[a]	2.59[a]	11.26[ab]	1944[ab]	1.84[a]	59.42[b]	324.1[ab]
	6%	189.1[ab]	2.24[a]	2.03[a]	13.26[ab]	2156[ab]	1.85[a]	57.68[b]	395.7[a]
	8%	205.0[a]	2.54[a]	2.29[a]	14.79[a]	2515[a]	1.82[a]	58.56[b]	424.1[a]
2014	0%	136.5[b]	1.93[a]	3.35[a]	9.87[a]	1541[c]	2.09[a]	91.31[a]	116.3[c]
	2%	157.8[ab]	1.79[a]	2.64[ab]	9.78[a]	1705[bc]	1.86[a]	79.30[ab]	204.0[b]
	4%	180.2[ab]	1.94[a]	2.39[b]	9.03[a]	1964[abc]	1.74[a]	68.05[b]	224.9[b]
	6%	178.6[ab]	1.77[a]	2.12[b]	9.17[a]	2118[ab]	1.89[a]	69.81[b]	307.1[a]
	8%	194.4[a]	1.88[a]	2.18[b]	11.02[a]	2304[a]	1.65[a]	67.28[b]	334.0[a]
	Biochar	868.7	13.25	16.75	467.8	10771	0.92	16.42	3578

Values present in figure are treatment means. Samples taken from control fertilizer treatment sub-plots.

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.

Biochar analysis completed in triplicate from three separate biochar samples and means presented in table.
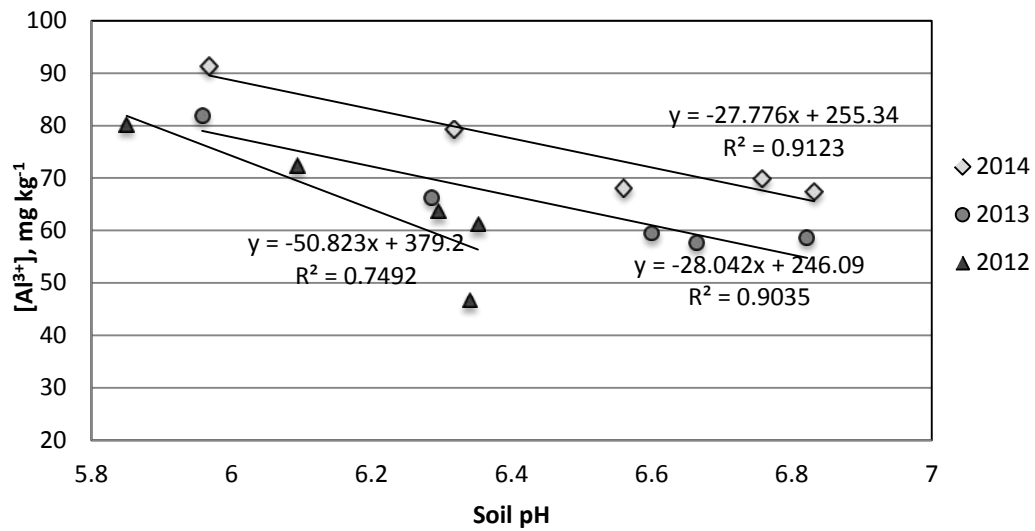
Figure 10: Soil pH at time of harvest and corresponding Al³⁺ concentration

The chart shows Soil pH (x-axis, 5.8 to 7) versus [Al³⁺], mg kg⁻¹ (y-axis, 20 to 100) for years 2014 (diamonds), 2013 (circles), and 2012 (triangles), with the following trend lines:

$$y = -27.776x + 255.34$$
$$R^2 = 0.9123$$

$$y = -50.823x + 379.2$$
$$R^2 = 0.7492$$

$$y = -28.042x + 246.09$$
$$R^2 = 0.9035$$
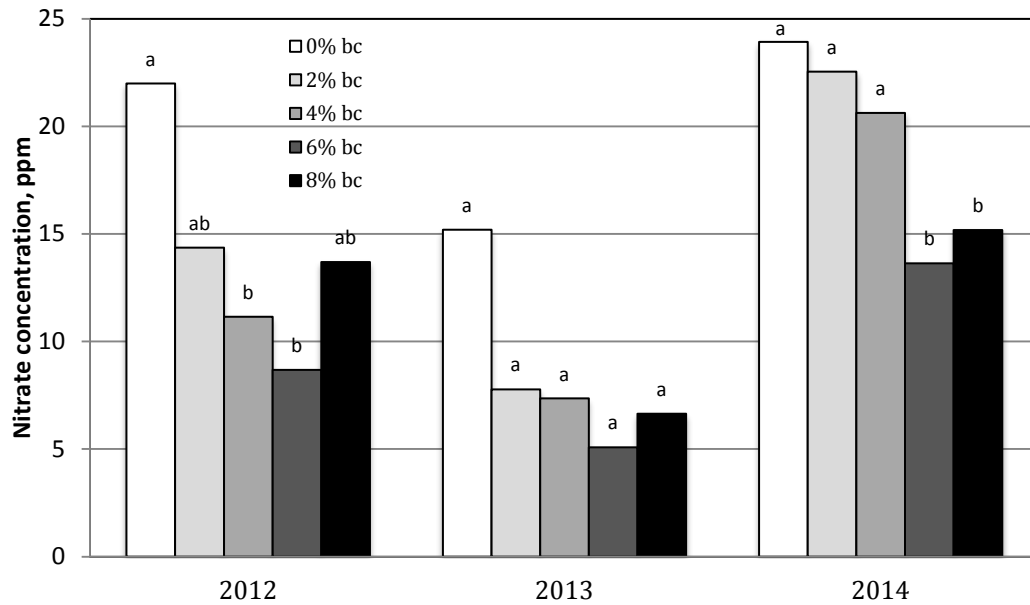
Figure 11: Pre-sidedress nitrate nitrogen concentration of biochar treatments at 0-20cm depth.

Values present in figure are treatment means. Samples taken before plots were split and additional fertilizer treatment was applied.

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.
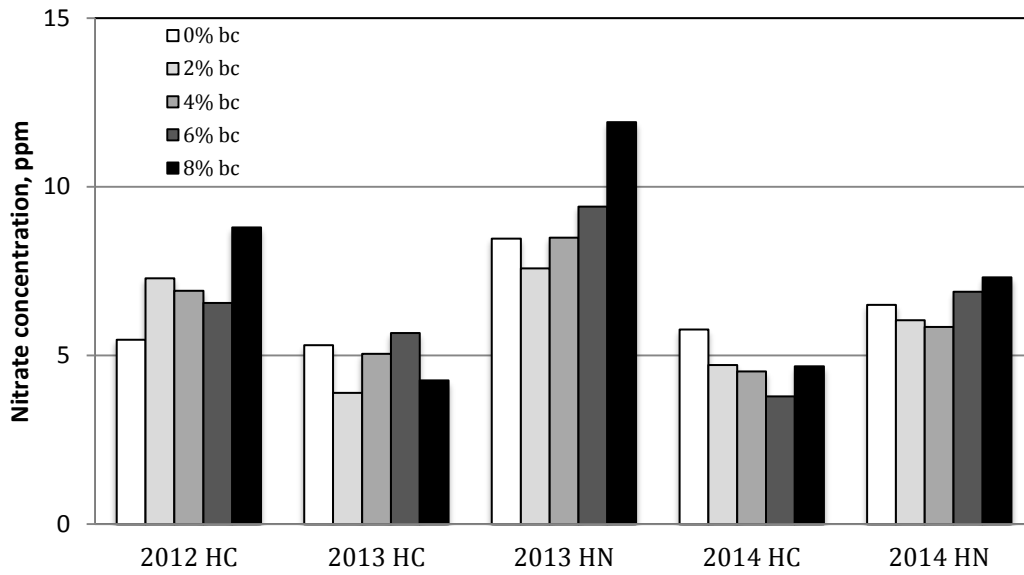
Figure 12: Soil nitrate concentration at time of sweet corn harvest at 0-20 depth

Values presented are treatment means. C is control (no added nitrogen fertilizer) and N is fertilizer treatment (56kg ha^{-1}).

No significant difference found at *P<0.05*.
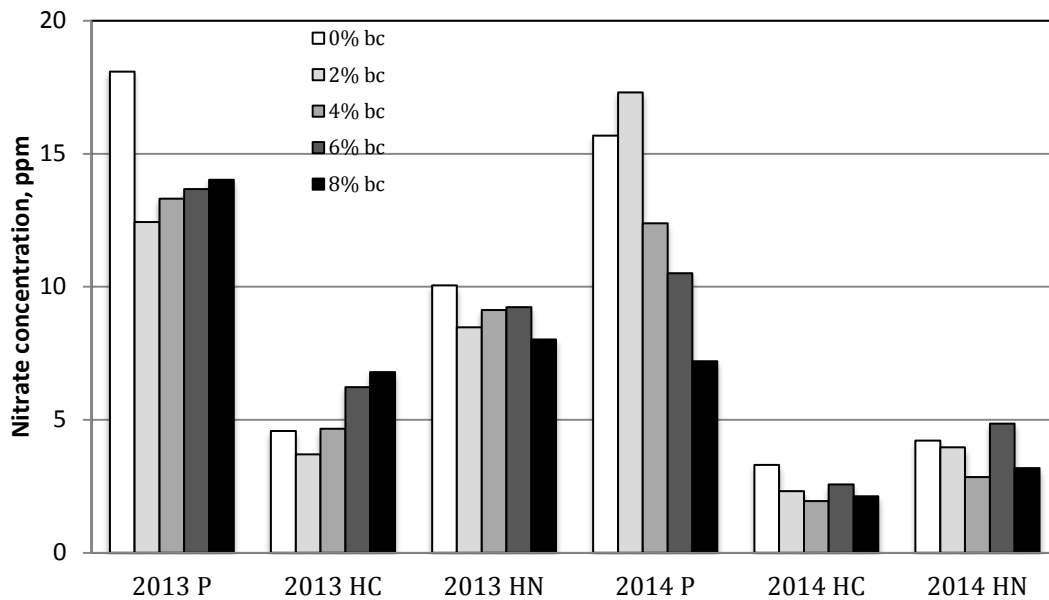
Figure 13: Soil nitrate concentration at PSNT and harvest, 20-40 cm depth.

P is PSNT timing, HC is at time of harvest control (no fertilizer), HN is at time of harvest with nitrogen fertilizer treatment (56kg ha^{-1}).

Values presented are treatment means.

No significant difference found at *P<0.05*.
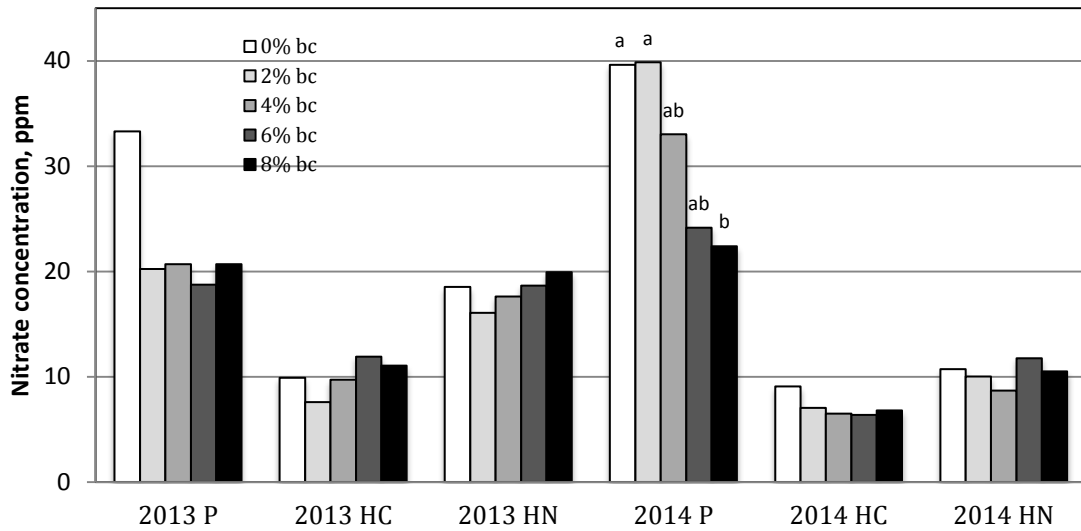
Figure 14: Total soil nitrate concentration at time of harvest, 0-40cm depth.

P is PSNT timing, HC is at time of harvest control (no fertilizer), HN is at time of harvest with nitrogen fertilizer treatment (56kg ha^{-1}).

Values presented are treatment means.

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.

CHAPTER 4

SWEET CORN YIELD AND QUALITY RESPONSE TO HARDWOOD BIOCHAR AMENDMENT

4.1 Introduction

The Connecticut River Valley has been known for it's rich agriculturally productive soils for over 100 years (Dorsey and Bonsteel, 1899). Historically, the area was known to grow tobacco and evidence of this history is still present today with a few tobacco farms surviving, and many tobacco barns still standing. More recently, sweet corn has become a very important local vegetable crop to Western Massachusetts. In Massachusetts, 422 farms had a total of 4985 acres in sweet corn production, which comprise approximately 1% of Massachusetts land. Nationally, Massachusetts is ranked 19th in total acreage of sweet corn production, which is significant considering it is the 44th state in total land area (USDA-NASS, 2014).

The field site under investigation abuts the Connecticut River and has been designated with an erosion factor (Kw) of 0.43. This is relatively high and of concern due to the high silt content of the field soil under investigation. Due to the proximity of the river and the potential for loss of surface soil, biochar is investigated for its potential ameliorating properties in the previous chapter. In this study, the effects of a low-grade, yet readily available biochar was investigated for its effects on the yield and quality of sweet corn to elucidate any potential negative effects on this locally valuable crop.

4.1.1 Field Site Characterization and History

A three-year field experiment was conducted at the University of Massachusetts Amherst Crops and Animal Research and Education Farm in South Deerfield, MA (42°28'37"N, 72°36'2"W). The soil in the research field is labeled as prime farmland and is a moderately well drained Winooski silt loam (coarse-silty, mixed superactive, mesic Fluvaquentic Dystrudept) with a high run-off class. The Kw value for this soil is 0.43, which is rather high in a scale from 0.02 to 0.69. The depth to water table is approximately 22-24 inches and mean annual precipitation at this site ranges from 37-51 inches (940-130mm). The mean annual temperature is 37 - 51° F (3 -10.5 ℃). The research site had previously been left fallow, but mowed consistently, for 2 seasons prior to use in this study.

4.2 Materials and Methods

4.2.1 Field Study Experimental Design

The research area consisted of twenty-five 3 meter x 6 meter plots with 1.5 meter buffers between neighboring plots. Initial field preparation included plowing and disking along with the application of 1 inch of compost (applied with manure spreader). The layout of the experiment was split-plot design with five replications. Main plots were allocated to five application rates of biochar; control-0%, 2%, 4%, 6%, and 8% by weight (equivalent to the rates of 0, 40.5, 81.1, 121.5, and 162.0 Mg/ha respectively) which was applied to the research plots by hand. Experimental plots were constant throughout the three years of study. Research plots were disked to incorporate the biochar into the top 15cm of soil by disc harrow. Sub-plots consisted of two nitrogen rates including 0 and 56 kg ha-1 in the

form of calcium ammonium nitrate (CAN), which was applied by hand at the V6-7 growth stage of corn in 2013 and 2014.

The biochar used in this study was derived from sugar maple wood and processed using a Missouri Kiln (maximum temperature of approximately 400° C) for use as hardwood lump charcoal. Biochar characterization is presented in the following section.

An early maturity sweet corn hybrid (*Zea mays L. cv Spring Treat)* was planted using a cone type distributor mounted on a double disc opening corn planter. An early variety of sweet corn, *Spring Treat*, purchased from Johnny's Selected Seeds (Winslow, ME) was used in this research study. *Spring Treat* is a short season hybrid variety of yellow sweet corn with cool soil vigor. Prior to planting each season, field plots were treated with glyphosate herbicide and soils was prepared with a disk harrow. Sweet corn was planted in three rows (0.75m spacing), centered in each plot at a rate of 60000 plants per hectare. A combination of *S*-metolachlor and atrazine (Bicep II Magnum, Syngenta Corp.) was used as pre-emergence herbicide. Corn plants were not irrigated at this location because rainfall is normally considered adequate.

4.2.2 Marketable Yield Analysis

In each year of this study, ears of corn were harvested from a 3-meter sample row within each plot. The total mass of quality ears was weighed and extrapolated to hectare production for marketable yield. If second ears were present and market quality they were harvested and included in marketable yield. In years 2012 and 2013, three ears from each plot were kept for further analysis. Ears were measured for length and unfilled kernel percentage (tipfill) as an indicator of quality. No significant differences in ear length and

percent tipfill were observed; therefore discussion of that data has been omitted. In the third year of this study, full plants were cut down to calculate the harvest index. Ears were separated from stalks and both were weighed and data recorded. All results were analyzed using the number of plants in the harvest area as a covariate using the GLM Procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

4.2.3 Corn Stalk Nitrate Testing

Each year, three corn stalks from every sub plot were harvested from the same sample area where ears were harvested for yield analysis. A six-inch section was cut and removed at the base of the stalk, just above the roots. These were dried in a forced air oven at low temperature. Once dry, tissue samples were ground, and 200mg of tissue powder was added to a 250 Erlenmeyer flask and was shaken in 40mL of acetic acid (2.0%) for 15 minutes. Tissue was filtered from the supernatant and then the supernatant was analyzed for nitrate by colorimetric determination using flow injection analysis (QuickChem 8000, LaChat Instruments, Loveland, CO). All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

4.3 Results and Discussion

In the pilot year of this study, sweet corn was not planted until the last week of July, which is nearly 60 days after the recommended planting time for this variety of sweet corn. This was due to a delay in the acquisition of the biochar and thus it caused a delay in the soil application and subsequent planting. This caused a significant delay in harvest resulting in unusually low yield values. On top of the delayed planting, a full replication (Block 3) of

yield values were disregarded due to the loss of product to raccoons. Yield values are still of interest in comparison amongst the biochar treatment levels and the control, but overall yield cannot be accurately compared to the typical regional yield.

In early June of 2013, the field location of this experiment received record amounts of rain totaling over 250 mm of precipitation, which is twice the norm for this location (average of 50 years). The timing of this rainfall caused significant under-germination in the planting that season. As a result, the field was re-planted the first week of July, and yield values were negatively affected due to the late timing of the successful planting. As with the yield in the pilot year of this study, overall yields cannot be compared to local yield averages. The region, as a whole, suffered yield losses up to 30% (Barry, 2013). However in 2014, the sweet corn was successfully planted on time at the then end of May and the yields reflect the full season of growth and are comparable with other locally grown sweet corn yields.

In the 2012 growing season, the addition of biochar to the prime agricultural lands used in this study showed minor yield improvement at an application rate of 2% (w/w) but decline with further increase of application rate to 4% or higher. The biochar treatment level of 2% corresponds to a rate of 40.5 t ha^{-1}of biochar. Crop yield increases due to application of wood biochar have been reported at rates ranging from 10 to over 60 t ha^{-1} (Steiner et al., 2010; Rondon et al., 2007; Yamato et al., 2006;), as well as decreases in yield (Chan et al., 2007; Gaskin et al., 2010). The 2% biochar treatment level increased the pH of an acid soil significantly, increased the availability and potentially the uptake of P, Ca, Mg, and K, while decreasing the available Al^{3+}. This is the likely cause of the slight increase in yield seen in both the 2012 and 2013 growing seasons, with no additional nitrogen fertilizer. This aligns with reported increased in nutrient availability with the addition of an

alkaline biochar to acid soils as reported by Steiner et al. (2007). However, in the third growing season (2014), yields were depressed at all biochar rates with no nitrogen fertilizer treatment, likely due to the relatively low nitrate concentrations in all treatment plots due to two prior seasons of sweet corn production with removal of biomass at the end of each season, effectively removing nitrogen from the field. During this time, only half of each main plot received nitrogen fertilization and it was at half the recommended rate for sweet corn in New England (UMass Extension, 2014). In the nitrogen-fertilized sub-plots, the 2% biochar treatment level once again rebounded and was statistically the same as the control yields.

Through all years and treatment levels, yield differences were small in comparison with those reported in tropical soils with decreased soil health and increased weathering (Lehmann et al., 2003; Van Zweiten et al., 2010). The soils in this study do not have significant fertility issues, thus it is logical that a biochar amendment in these soils would have muted effects on the yield of field crops.

Nitrogen uptake by the corn plants has been long studied as an indicator of sufficient and or excessive nitrogen nutrition in field corn (Scarseth, 1943; Hay et al., 1953; Fox et al., 1989). Corn stalk testing has been limited however in its application to sweet corn (Heckman et al., 2002). Stalk nitrate concentrations of sufficiently or over-fertilized sweet corn, reported by Heckman et al. (2002) ranged from 6.1 g kg^{-1} to 20.9 g kg^{-1} in the varieties *Calico Belle, Brilliance Film Coat, Sensor and Silverado.* The variety of sweet corn accounted for the greatest variation in stalk nitrate concentration and therefore interpretation of a corn stalk nitrate test on sweet corn is difficult with new varieties, such as the short season variety *Spring Treat*, used in this experiment.

The corn stalk nitrate testing of the corn in this study showed inconsistent data with no clear trend in data except for the 2014 growing season. In both the fertilized and unfertilized treatments there was an overall decrease in corn stalk nitrate concentration as the biochar application rate increased. In the fertilized plots, the mean stalk nitrate concentration dropped over 25% from the control stalk nitrate concentration of 4.1 g kg^{-1} to 2.7, 3.1, and 2.9 g kg^{-1} the 4, 6, and 8% application rates, respectively. Although there was less nitrate storage in the corn stalk of the higher biochar treatment corn, a similar decline was not seen in the yield of those same treatment levels. The drastically lower stalk nitrate concentrations with minimal yield decline may indicate that for this cultivar of sweet corn stalk nitrate concentrations of greater than 3g kg^{-1} may correspond to sufficient nitrogen nutrition. Further trials with greater nitrogen fertilization rates would be necessary to determine the range of corn stalk nitrate concentrations that indication optimal growth and the corresponding nitrogen fertilization rates for *Zea mays L.* cv Spring Treat.

One additional aspect to consider in the nitrogen availability and uptake of corn is the soil pH and its effect on the nitrifying bacteria population. It has been long documented that an increase in the soil pH can increase the rate of nitrification in soils (Paavolainen and Smolander, 1998). The lack of a significant decline in sweet corn yield with lower stalk nitrate concentration may indicate a steady, but increased pool of available nitrate from nitrifying bacteria present in the biochar-amended soils. Paavolainen and Smolander reported an increase in overall nitrification in clear-cut forest plots as pH increased due to an increase in the community of nitrifiers (1998). Additional bacterial community analysis is necessary to assess this effect in the current study.

4.4 Conclusion

Biochar yield was not significantly impacted by the addition of biochar to the soil. There were non-significant yield increases with the 2% biochar application rate when both no additional nitrogen amendment and 56kg ha^{-1} of nitrogen in the form of calcium ammonium nitrate (CAN) was applied. At biochar levels greater than 2% sweet corn yields were inconsistent, yet generally declined to below-control levels. The 2% biochar application rate may have promoted growth with the increase in available Ca, Mg, P and K without negatively impacting the nitrogen uptake of the corn plant, as indicated by the corn stalk nitrate concentrations. Overall, nitrogen fertilization caused a slight decline in yield, likely due to increased foliar growth over ear production.

Considering the lack of major growth retarding deficiencies in the soil, no more than 2% biochar application rate would be recommended for application to this field site.

Figure 15: Sweet corn (*Zea mays L.* cv Spring Treat ®) rows, mid-season. July, 2014.

 (Johnny's Seed Co. Maine).
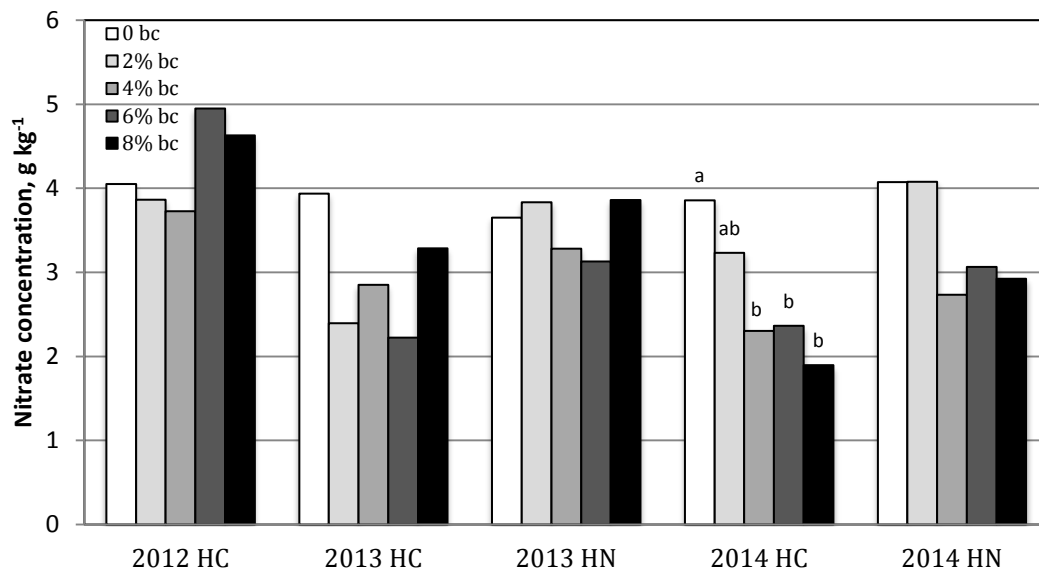
Figure 16: Corn stalk nitrate test conducted at time of harvest.

Lower 6 inches section of stalk harvested and dried. Clustered by year and fertilizer treatment.

HC is control (no fertilizer), HN is with nitrogen fertilizer treatment (56kg ha^{-1}).

Values presented are treatment means.

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.
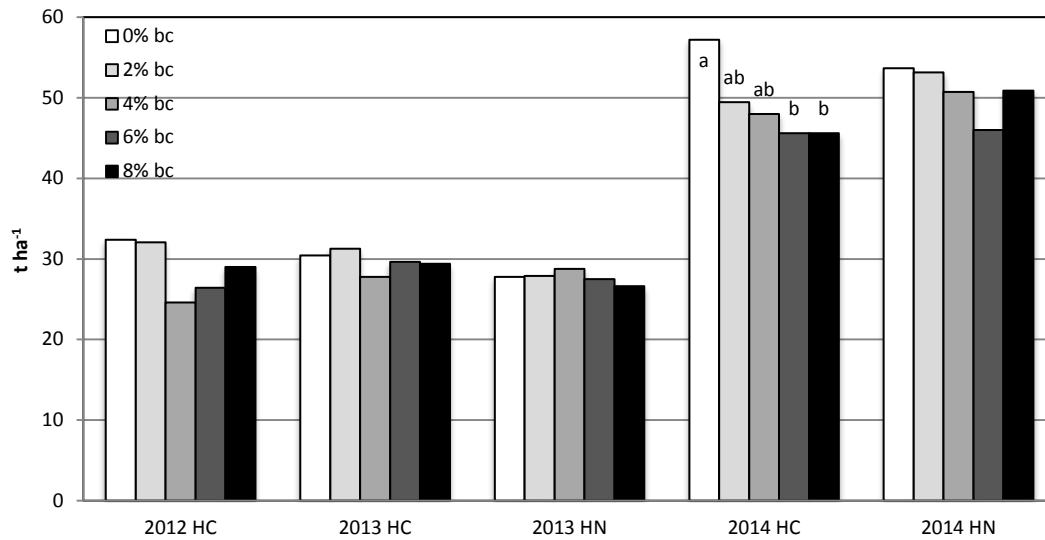
Figure 17: Marketable yield of sweet corn (*Zea mays L.*).

Includes all 1st and 2nd ears at least 6 inches in length and of high quality.

Values presented are lsmeans and adjusted using the covariate (#plants).

Means followed by the same letter are not significantly different at *P<0.05* as determined by Tukey's HSD.

CHAPTER 5

NEMATODE COMMUNITY SHIFTS IN RESPONSE TO HARDWOOD BIOCHAR APPLICATION

5.1 Introduction

Soil biota is often overlooked when soils (especially agricultural soils) are assessed for their soil health and quality. Often farmers are assessing the nutrient content and physical structure of the soil to determine the potential need for any soil amendment or structural working, such as tilling. Recently, soil health and quality have begun to include an assessment of the soil biological component. Unfortunately, this aspect of soil health is very difficult to assess with simple sampling and diagnostic tools unlike assessing soil pH or calcium content. The soil biotic component includes a vast array of organisms: from larger macrofauna such as earthworms, ants, and termites, microfauna such as arthropods, to the microscopic bacteria and fungi.

Nematodes are one of the most numerous phyla of animals on the planet and have a significant impact on the soil ecosystem (Coleman and Wall, 2015). The nematode population itself has a significant impact on nutrient cycling and decomposition in most eco-systems, and is of special interest in agroecosystems where nutrient availability within the soil affects crop production and the overall economic stability of farms (Ingham et al., 1985). As bacteria (and fungi) begin to mineralize organic substrates in the soil, nutrients such as nitrogen are immobilized within the bacterial cell. With the presence of bacteria-feeding nematodes, those nutrients are released and cycled back to the soils through digestion (Trofymow et al., 1983, Ingham et al., 1985) and then may potentially be available

for plant uptake. Trofymow et al. (1983) found that when bacteria populations were grazed by bacteria-feeding nematodes, there was greater C and N mineralization in microcosm studies.

Complementary to microbial community analysis, nematode community structure can also be a viable bio-indicator of environmental health and useful as they are generally easy to sample, they are relatively stable communities in the soil and are the most numerous animal on the planet (Ferris and Ferris 1974; Bongers 1990; Visser and Parkinson; 1992; Bongers and Bongers 1998; Bongers and Ferris, 1999; Neher, 2001). Yet, nematode response to biochar amendment has been largely overlooked and under-reported in literature (Lehmann et al., 2011; Ameloot et al., 2013). Nematodes vary in their responses to pollutants and disturbances in their environment as the analysis of these responses in community structure can be a powerful tool for in situ assessment of soil health.

Nematode identification is helpful for biological assessment of soils for many reasons including these expressed by Bongers and Bongers (1998): (a) nematodes occur everywhere where decomposition takes place, (b) their morphology reflects feeding behavior, (c) of their interactions with other soil biota, (d) of their food-specificity, (e) they have a short response time, (f) they are easily isolated from the substrate and (g) genus identification is relatively simple. Identification of nematode morphological features, including mouthparts, allows for identification of primary food sources for each identified genus as well. Primary feeding types of nematodes include plant feeders also called plant parasitic (PP), fungal feeders (F), bacterial feeders (B), predatory feeders (P), and omnivorous feeding (O) (Yeates et al., 1993). While identification and key community indices are relatively easy to assess, characterizing the ecological shifts and relationships of nematodes in a soil environment is far less so.

In order to more easily use nematodes as environmental indicators, they must be identified and organized into their feeding groups (Yeates, 1984) as well as assigned colonizer-persister (CP) values, which represent their reproductive strategies (Bongers, 1990; Bongers and Bongers, 1998; Ferris et al., 2001; Yeates et al., 1993). Colonizers reproduce quickly and in large numbers when the environmental conditions are healthy and undisturbed, while maintaining resistance to environmental disturbance. In contrast, persister families of nematodes reproduce in much smaller numbers and are rarely the dominant species in any given ecosystem. Persisters are also more susceptible to ecological disturbance (Bongers, 1990). The CP value ranges from 1-5, which 1 representing extreme colonizer behavior and 5 representing extreme persister behavior. The use of feeding groups and CP values is vital in assessing soil ecosystems and food webs. It is so far impossible to determine the effect that a single species of nematode has on its environment, therefore, larger groupings and assessment scales are necessary for ecosystem evaluation (Bongers and Bongers, 1998). Although, species can be individually identified using identification keys which have become much more comprehensive.

Several indices have been developed to try and characterize the population of nematodes within a sample ecosystem, often referred to as nematode assemblages (Bongers, 1990). The Shannon diversity index (Shannon, 1948) calculates the diversity within a sample and allows for between sample comparisons using the following equation:

$$H' = \sum p_i \ln p_i$$

where p_i is the proportion of individuals in the (i)th family. The maturity index (MI) developed by Dr. Tom Bongers is useful when comparing samples before and after environmental changes or disturbances, or across a treatment gradient. It is less useful

when analyzing difference soil types and environments for simple comparisons. Lower MI

values indicate greater concentration of colonizers, which are more resistant to stress and

disturbance to the ecosystem. Higher MI values indicate more stable communities with

greater numbers of persister families. The maturity index is calculated as follows:

$$MI = \sum_{i=1}^{n} v(i) * f(i)$$

where v(i) is the cp value of the identified family, and f(i) is the frequency of that in the

sample (Bongers, 1990). This index is calculated using only non-plant parasitic nematodes.

A plant parasitic MI can also be calculated in the same manner but is referred to as PPI.

While the maturity index provides a simplified tool to compare nematode

communities in related soils, or experimental treatments, it does not offer detail into the

structure of the community. Additional nematode community indices (Enrichment,

Community and Structural Indices) have been developed to evaluate the differences among

communities while trying to account for changes in specific taxa that indicate the

abundance of sub-groups of nematodes, called functional nematode guilds (Ferris et al.,

2001).

Biochar and its impacts on the soil nematode population have been largely under-

reported in the scientific literature. In the most recent studies that have been reported, only

the ratio of free-living nematodes to plant parasitic nematodes as affected by poultry-litter

biochar has been evaluated (Rahman et al., 2014). It was reported by Rahman et al., that the

addition of biochar to a vineyard soil in New South Wales, Australia lowered the plant-

parasitic nematode population the greatest. This work was the second such study to report

the reduction of plant parasitic nematode populations in biochar amended field soils. In an

earlier study, biochar produced from wheat straw was shown to decrease the overall plant

parasitic nematode abundance when applied at levels of 12 and 48 t ha^{-1} as well as increase

the fungivore population with no significant effect on overall nematode abundance across

biochar application rates (Zhang et al., 2013). These are the only two studies to date that

take a closer look at the effects of biochar on the nematode community in field trials. In a

reported pot trail, an increase in one species bacterial-feeding nematodes (*Acrobeloides* sp.)

was reported when soils were amended with 1 and 2% grass biochar (Fox et al., 2014). This

particular bacteria-feeding nematode has a CP value of 1, indicating that it is a fast

reproducer and likely able to increase population due to an increase in overall bacteria

population in the biochar amended pots.

The relative abundance of nematodes, their diverse feeding habits, and their

relatively stable life cycle allow researchers to utilize nematode communities as valuable

indicators of soil and ecosystem health (Ferris and Ferris 1974; Bongers 1990; Bongers and

Bongers 1998; Bongers and Ferris, 1999; Neher, 2001). In this study, the effect of hardwood

biochar on the overall nematode abundance, trophic group abundance, family richness,

Shannon diversity (Shannon, 1948) and maturity indices will be evaluated.

5.1.1 Field Site Characterization and History

A three-year field experiment was conducted at the University of Massachusetts

Amherst Crops and Animal Research and Education Farm in South Deerfield, MA

(42°28'37"N, 72°36'2"W). The soil in the research field is labeled as prime farmland and is a

moderately well drained Winooski silt loam (coarse-silty, mixed superactive, mesic

Fluvaquentic Dystrudept) with a high run-off class. The Kw value for this soil is 0.43, which

is rather high in a scale from 0.02 to 0.69. The depth to water table is approximately 22-24 inches and mean annual precipitation at this site ranges from 37-51 inches (940-130mm). The mean annual temperature is 37 - 51° F (3 -10.5 ℃). The research site had previously been left fallow, but mowed consistently, for 2 seasons prior to use in this study.

5.2 Methods and Materials

5.2.1 Field Study Experimental Design

The research area consisted of twenty-five 3 meter by 6 meter plots with 1.5 meter buffers between neighboring plots. Initial field preparation included plowing and disking along with the application of 1 inch of compost (applied with manure spreader). The layout of the experiment was randomized complete block with five replications. Plots were allocated to five application rates of biochar; control-0%, 2%, 4%, 6%, and 8% by weight (equivalent to the rates of 0, 40.5, 81.1, 121.5, and 162.0 Mg/ha respectively) which was applied to the research plots by hand. Experimental plots were constant throughout the three years of study. Research plots were disked to incorporate the biochar into the top 15cm of soil by disc harrow.

The biochar used in this study was derived from sugar maple wood and processed using a Missouri Kiln (maximum temperature of approximately 400° C) for use as hardwood lump charcoal. Biochar characterization is presented in the following section.

An early maturity sweet corn hybrid (*Zea mays L. cv Spring Treat,* Johnny's Selected Seeds, Winslow, ME*)* was planted using a cone type distributor mounted on a double disc opening corn planter. Prior to planting each season, field plots were treated with glyphosate

herbicide and soils was prepared with a disk harrow. Corn plants were not irrigated at this location because rainfall is normally considered adequate.

5.2.2 Sample Collection and Nematode Identification

Soil samples were taken in July 2013 from the highest and lowest (8 and 0% respectively) biochar treatments in all field replications. Five sub-samples (0-20cm) were taken from each sampled plot within the rows of corn and combined. Soil for nematode samples was stored at 4°C until processing. Nematodes were extracted from the soil using the modified Cobbs sifting and gravity method followed by centrifugation and sugar flotation (Cobbs, 1913; Neher et al., 1999; 2010). Three technical replicates for each plot were completed. Nematode samples were added to a counting dish, identified and counted under an inverted compound microscope. Nematodes were identified to genus level using published identification keys (Goodey, 1963; Mai and Lyon, 1975; Tarjan et al., 2014). Nematodes were assigned to their appropriate trophic groups and colonizer-persister values (Yeates et al., 1993; Neher et al., 2004; Okada et al., 2005). The maturity index (MI), plant parasitic maturity index (PPI), taxon richness, ratio of fungivore to bacteriavore nematodes, and Shannon's Diversity and Simpson indices were calculated for each technical replicate for each plot (Shannon, 1948; Bongers et al., 1997; Ferris et al., 2001; Neher et al., 2004). All results were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc. Cary, NC). Means separated by Tukey's HSD at significance level of $p < 0.05$.

5.3 Results and Discussion

There are many management factors in agricultural soils that can affect the nematode population. In turn, nematode communities also tend to react and adapt to these management practices in a predictable manner. Comparing and identifying shifts in the nematode community structure can aid is assessing how the agricultural practices are affecting the soil, microbial and micro-faunal communities. The development of nematode trophic groups, colonizer-persister classification and maturity indices have allowed easier comparison amongst soil types, crop rotation, fertilizer treatments and other agricultural management practices (Bongers, 1990; Yeates et al., 1993; Neher et al., 1995; Bongers et al., 1997; Neher and Olson, 1999; Fiscus and Neher, 2002).

In this study, 19 genera of nematodes were identified from 14 different families. Nematode communities were dominated by bacteria-feeding nematodes in both the control and the 8% biochar treatment. All nematode families identified in the biochar-amended soil were also identified in the control soil. However, the control soil contained two families of nematodes that were not identified in the biochar-amended soil samples. Those families include the predatory Mononchidae and Tripylidae. The biochar plots contained one family of nematodes not found in the control plots, the plant parasitic Trichodoridae family. The bacteria-feeding family, Rhabditae, was the predominant family identified in both the treated and untreated soil. The predatory nematode family, Discolaimidae, was also found in high numbers in both soil types. Two nematode families, Tylenchidae and Anguinidae, were also identified in both the biochar-amended and control soil. Both of these nematode families contain both fungal feeders and plant parasitic trophic groups. In order to better evaluate trophic groups and maturity indices, half of each family was classified as fungi-feeding and half as plant parasitic nematodes. This is a reasonable solution to the difficulty

90

in assigning tropic groups to nematode families that have varying feeding habits (Yeates and Bongers, 1999).

Overall, the presence of biochar at a high level (8%, 162 t ha^{-1}) decreased the abundance of nematodes, although non-significantly. This decrease is accounted for mainly through a decrease in the number of bacteria-feeding and plant parasitic nematodes. Interestingly, the abundance of predatory nematode was higher in the biochar-amended soil. The Shannon Diversity index (H') was significantly lower in the biochar-amended soil (*p>0.001*), but the taxa richness of the biochar-amended soil was not significantly different from the control. The lower diversity index may be attributed to the Shannon index equation itself. The Shannon index has been reported to heavily depend on abundant taxa in the community (Yeates and Bongers, 1999), therefore due to the significantly lower abundance of the most dominant family (*Rhabditae*) the diversity index decreased in the biochar-amended soil.

The maturity index (MI) was significantly higher (*p>0.01*) in the biochar amended field soils, with a mean value of 2.37 as compared to 1.97 for the control soil. Although all plots were managed in the same exact manner, the biochar-amended soils had a greater abundance of taxa with C-P values of 5, indicating greater numbers of extreme persister nematodes. Persisters are more susceptible to ecological disturbance and tend to reproduce at slower rates (Bongers, 1990). The dominance of bacterial feeding nematodes and greater numbers of predators and omnivores in the biochar-amended soils may indicate that there is greater stability within the eco-system. If the taxa abundance indicate more overall evenness, that can indicate more internal regulation as there are greater numbers of predators to keep other colonizer taxa from over-populating the ecosystem (Ferris and Bongers, 2009).

The addition of significant amounts of Mg, Ca, K, and Mn in the biochar-amended soils may have an effect on the nematode community. Significant increases in predatory nematodes with a reduction in plant feeding nematodes with the addition of several heavy metals to the soil have been reported (Bardgett et al., 1994). However, the addition of wood ash, which can contain very high levels of calcium as well as significant amounts of Mg, K, Al, P and other heavy metals, to one microcosm study caused an increased in overall abundance and bacteria feeding nematodes (Liiri et al., 2002).

The fungivore to bacteriavore ratio declined, although not significantly, in the biochar-amended soils. This is likely due to several potential changes caused by the application of biochar. First, the pH shifts towards neutral with the addition of an alkaline biochar such as the one used in this study. The increase in pH will favor bacterial populations over fungal populations, thus creating a more competitive environment for fungi-feeding nematodes and lowering the population (Liiri et al., 2002, McCormack et al., 2013). In their 2013 review, McCormack et al., predicted a decline in fungal abundance and increase in bacterial abundance with the application of biochar and its associated pH increase. While the lowered fungal-feeding nematode population in this study's biochar amended soils may indicate lower fungal abundance, there was no associated increase in bacteria feeding nematodes indicating no increase in bacterial abundance. The increase in predatory nematodes, however, does indicate greater predatory nematode grazing. Also, it has been shown that P availability in soils is negatively correlated with abundance of mycorrhizal fungi as well as other soil fungi (Menge et al., 1978; Nagahashi et al., 1996). Lastly, a negative correlation between soil calcium and magnesium concentration and the percentage of fungal feeding nematodes present in soil has been reported (Wang et al., 2004). These factors can all contribute to lower fungal abundance, explaining the lower

fungivore to bacteriavore ratio in the biochar amended soils. To better assess this reasoning, future work regarding fungal abundance is necessary.

5.4 Conclusion

Management practices have a significant effect on the nematode population in agricultural soils. The addition of biochar to soils that are annually prepared by disk-harrow and planted under sweet corn has allowed for greater succession into a more temporally stable assemblage as indicated by the larger maturity index, and lower population od colonizer nematodes. However, an application rate of 8% biochar by weight (160 t ha^{-1}) is not recommended for most agricultural soils. A smaller application rate of 2% is recommended, but may not impact the nematode community significantly at that low rate. Further investigation of nematode populations at lower biochar application rates is necessary to investigate this further.

Table 6: Nematode taxa identified, taxon counts, feeding habits, CP values, guild and abundance per biochar treatment.

Family	Feeding Habit	CP Value	Guild	Biochar	
				0%	8%
Diplogasteridae	B	1	B1	32.8[a]	19.4[b]
Monhysteridae	B	1	B1	11.9[a]	5.7[b]
Rhabditae	B	1	B1	145.9 [a]	113.1[a]
Plectidae	B	2	B2	32.7[a]	27.3[a]
Nordiidae	O	4	O4	15.6[a]	16.4[a]
Tripylidae	P	3	P3	0.1[a]	0[a]
Mononchidae	P	4	P4	0.1[a]	0[a]
Discolaimidae	P	5	P5	48.9[a]	69.2[a]
Tylenchidae	PP/F	2	H2/F2	1.0[a]	0.1[a]
Anguinidae	PP/F	2	H2/F2	17.5[a]	10.1[b]
Dolichoridae	PP	3	H3	1.5[a]	0.5[a]
Hoplolaimidae	PP	3	H3	0.1[a]	0.3[a]
Pratylenchidae	PP	3	H3	15.3[a]	7.4[b]
Trichodoridae	PP	4	H4	0[a]	0.1[a]

B= bacteriavore; O= omnivore; P= predator; PP= plant parasite; F= fungivore.

Means with the same letter indicate no significant difference at *p=0.05*.

Table 7: Nematode counts by trophic group

Indices	Biochar	
	0%	8%
n	323.3	269.7
B	223.3	165.6*
F	9.2	5.1**
O	15.6	16.4
P	49.1	69.2
PP	26.10	13.37**
FF:BF	4.25	2.76
Richness	10.60	10.40
H'	1.97	1.76**
λ	0.19	0.24**
MI	2.21	2.38**
PPI	3.51	3.60

B= bacteriavore; O= omnivore; P= predator; PP= plant parasite; F= fungivore

n = abundance per 5 grams of soil.

FF:BF = fungivore to bacteriavore ratio

H' = Shannon Diversity Index

λ = Simpson Index

Calculated maturity indices (MI) and plant parasitic maturity indices (PPI) for field plots include CP values 1-5.

* Significantly different from control, *p<0.05*.

** Significantly different from control, *p<0.001.*

	A	B	C	D	E	F
4				2.459	1.969	
3		2.237			2.467	
2	3.063		2.131			1.739
1			2.015		2.145	1.500

Figure 18: Spatial arrangement of field plots and MI values.

Shades plots are 8% biochar treatment levels, and non-shaded plots are the control.

CHAPTER 6

SOIL BACTERIAL COMMUNITY RESPONSE TO HARDWOOD BIOCHAR AMENDMENT

6.1 Introduction

Soil microbial communities are complex and the interaction of these communities with nutrient cycling is even more so. There are many taxa involved with the decomposition of organic material including bacteria, fungi, nematodes and more. The addition of biochar to soils can affect these microbial communities and their role(s) in the soil significantly (Warnock et al., 2007; Anderson et al., 2011; Zimmerman et al., 2011). Many theories have been discussed and presented as possible explanations for the changes to the soil microbial community. For example, the same varied pore space that is found in biochars and increases the WHC can also affect the biota within the soil. One theory for increased microbial activity in biochar-amended soils is that the pore spaces give bacteria and fungal hyphae a physical refuge from predation (Warnock et al., 2007). Protozoa and nematodes that feed on these organisms are much larger in size and cannot physically enter the pore space within biochar particles. The average sizes of soil bacteria and fungal hyphae range from 1 to 4 μm and 2 to 64 μm, respectively, while a typical protist could be 100 μm. Simply analyzing the size differences and comparing them to a typical biochar pore space indicates that this is a possible mechanism for increased microorganism diversity and activity in biochar-amended soils (Glaser, 2007; Warnock et al., 2007). The pores of biochar can also serve as a habitat for fungal hyphae (Thies and Rillig, 2009) and can then sporulate within the micropores leading to greater rates of mycorrhizal infection (Lehmann and Rondon, 2005).

Another theory regarding changes to microbial communities within biochar amended soils is that biochar may ameliorate the presence of specific compounds, such as volatile organic compounds (VOCs) that can both inhibit and enhance microbial processes such as mineralization and reproduction (Graber et al., 2010; Spokas et al.; 2011b). Elad et al (2010) also suggests that the shifts in microbial diversity and population are due to changes in the presence of biocidal agents in the biochar. These changes can be potentially harmful if they reduce the nitrifying communities or beneficial if the biocidal agent present (indirectly) improves plant stress resistance (Elad, 2010).

Or potentially, changes in microbial activity, within a biochar-amended soil, are the result of changes to the pH of the soil. If acidic, the soil pH can be shifted towards neutral with the addition of most biochars. A more neutral pH is conducive to increased bacterial growth. As microbial populations are most affected by the pH of their environment, biochar applications have the potential to significantly shift the make-up and the growth of the community (Atkinson et al., 2010).

Unfortunately, a large and healthy microbial community may temporarily lower the nutrient availability in a soil recently amended with biochar. Nitrogen immobilization is often observed in freshly amended soils due to the high C:N ratio of the added biochar (Lehmann, et al., 2003; Steiner et al., 2008). Bacteria will utilize all available nitrogen while trying to degrade high carbon compounds making nitrogen temporarily unavailable to plants for uptake (Warnock et al., 2007). This is a temporary effect and nitrogen levels often rise again shortly thereafter. Over time, the microbes in the soil will begin to oxidize the surfaces of the biochar particle increasing the negative charge density and therefore continually increasing the CEC of the biochar-amended soil. However, although surface oxidation may occur, the biochar itself is resistant to mineralization by the soil microbes.

Kuzyakov et al., (2014) for that after almost 4 years, less than one percent of the carbon (labeled with ^{14}C) from an initial biochar application was recovered in microbial biomass. Studies have also reported increases in mycorrhizal fungi in the presence of charcoal (Lehmann et al., 2006). The long-lasting changes in both physical and chemical properties of a soil amended with biochar are causing shifts in the microbial communities of these soils (Kolton et al., 2011).

When the environment in which these communities inhabit changes rapidly, microbes can respond in varying modes of survival with some successful and some unsuccessful (DeAngelis et al., 2010). Thus, rapid environmental changes may cause shifts in the microbial community composition. The shifts in microbial community are often assessed using molecular analysis of 16S rRNA, as culture-based identification vastly underestimates population diversity as culturable bacteria are a mere fraction of the total bacteria present (Leff et al., 1995; Griffiths et al., 2000). However, due to the co-extraction of humic acids and other inhibitory substances, the soil microbial community can be challenging to assess in this manner. Recent advances in DNA sequencing, such as Quantum insights into Microbial Ecology (QIIME) and high-throughput sequencing like the MiSeq (Illumina, San Diego, CA) , are revolutionizing the analysis of microbial ecology (Caporaso et al., 2010a; Caporaso et al., 2012). QIIME is open-source software that aids in interpretation of raw sequencing data. It allows for within- and between- sample diversity analysis and can aid in building phylogenetic trees (Caporaso et al., 2010b). Basespace, a cloud based analytical site and Illumina's answer to QIIME, also delivers a free and user-friendly interface in which distinct applications are available for a wide range of analytical techniques. These apps are free to use and include 16S Metagenomics analysis, which can be

used to determine microbial community changes at all taxonomic levels, based on the millions of base-pair reads using one of the Illumina sequencing platforms.

Sequencing of the 16S rRNA gene (or regions thereof) for prokaryotes and 18S for eukaryotes has moved to the front of microbial identification due to the ubiquitous nature in bacteria (and archaea) and also due the highly conserved and variable regions of these genes. The genes for 16S rRNA region of microbial ribosomes have changed very little over time. Sequencing of these changes allow for bacteria phylogenetic and taxonomic identification. RNA itself can be used in microbial community studies. RNA is an important indicator in environmental samples of the active microbial community, as rRNA and mRNA are generally indicators of 'functionally active microbial populations' (Torsvik et al., 2002). The rRNA concentration can indicate the growth of that specific bacterium (Rastogi et al., 2004).

It is important to note that in order to identify microbes at the genera and potentially species levels, there must be access to a large database of complete nucleotide sequencing (i.e. GenBank and ARB Silva). Recognized taxa of bacteria are rapidly expanding with faster, deeper and less expensive sequencing options. While there is a very large database of 16S rRNA data, identification to the species level is poor. For example, using 16S rRNA sequencing, *Pseudomonas florescens*, which excretes a pigment that can chelate iron is not distinguishable from *P. jessenii*, a phosphate-solubilizing bacterium (Janda and Abbott, 2007). Small differences in the 16S rRNA sequence are difficult to identify and recently diverged species may appear as though they are the same species. Or in the case of *Aeromonas veronii*, this bacterium has heterogeneity (difference up to 1.5%) within its own 16S rRNA, making it difficult to identify and catalogue (Janda and Abbott, 2007). Dunbar et al. (2002) reported using models, that to document just 50% of the species in a soil sample

would need a sample size of 16,000 – 50,000 individuals. Also, after analyzing clone libraries, Dunbar et al. found that over 50% of the total clones in the each of the four libraries came from just a few bacterial divisions, namely acidobacterium and proteobacteria.

There has been significant work towards building a set of soil bacterial and fungal primers for PCR quantification (qPCR). Quantitative PCR gives real-time quantitative analysis of the presence of bacterial and fungal groups through fluorescent output of a marker molecule. As amplification occurs, that fluorescent signal is also amplified and the presence of certain bacteria is detected. This is particularly applicable to soil microbial ecology, due to the high speed and input of qPCR. If the primers used are narrowed down to be more specific to your sample content then increase information can be retrieved. Fierer et al. (2005) used a set of primers that works specifically with the soil microorganisms they were interested in identifying. Using targeted primers, the relative abundance of taxonomic groups within different soil samples can be assessed. Just as DNA extraction can inherently bias data towards certain groups, the same can occur with qPCR. The relative abundance between soil samples is valuable data, however the abundance within a soil sample may not be an accurate value. There are many soil bacterial groups or members within a group that are not targeted with qPCR primers (Janda and Abbott, 2007).

Unfortunately, studies looking at the shifts in microbial communities at a high taxonomic level are few and far between. Chen et al. (2015) used pyrosequencing techniques to assess changes in the communities of bacteria most associated with nitrogen cycling in the soil. Nitrogen fixing, nitrifying, and denitrifying bacterial genera were assessed in rice paddy fields with biochar soil amendment; however, no clear and consistent effects were seen (Chen et al., 2015).

6.1.1 Field Site Characterization and History

A three-year field experiment was conducted at the University of Massachusetts Amherst Crops and Animal Research and Education Farm in South Deerfield, MA (42°28'37"N, 72°36'2"W). The soil in the research field is labeled as prime farmland and is a moderately well drained Winooski silt loam (coarse-silty, mixed superactive, mesic Fluvaquentic Dystrudept) with a high run-off class. The Kw value for this soil is 0.43, which is rather high in a scale from 0.02 to 0.69. The depth to water table is approximately 22-24 inches and mean annual precipitation at this site ranges from 37-51 inches (940-130mm). The mean annual temperature is 37 - 51° F (3 -10.5 ℃). The research site had previously been left fallow, but mowed consistently, for 2 seasons prior to use in this study.

6.2 Materials and Methods

6.2.1 Field Study Experimental Design

The research area consisted of twenty-five 3 meter x 6 meter plots with 1.5 meter buffers between neighboring plots. Initial field preparation included plowing and disking along with the application of 1 inch of compost (applied with manure spreader). The layout of the experiment was randomized complete block design with five replications. Plots were allocated to five application rates of biochar; control-0%, 2%, 4%, 6%, and 8% by weight (equivalent to the rates of 0, 40.5, 81.1, 121.5, and 162.0 Mg/ha respectively) which was applied to the research plots by hand. Experimental plots were constant throughout the three years of study. Research plots were disked to incorporate the biochar into the top 15cm of soil by disc harrow.

The biochar used in this study was derived from sugar maple wood and processed using a Missouri Kiln (maximum temperature of approximately 400° C) for use as hardwood lump charcoal. Biochar characterization is presented in the following section.

An early maturity sweet corn hybrid (*Zea mays L. cv Spring Treat,* Johnny's Selected Seeds, Winslow, ME) was planted using a cone type distributor mounted on a double disc opening corn planter. Prior to planting each season, field plots were treated with glyphosate herbicide and soils was prepared with a disk harrow. Sweet corn was planted in three rows (0.75m spacing), centered in each plot at a rate of 60000 plants per hectare. A combination of *S*-metolachlor and atrazine (Bicep II Magnum, Syngenta Corp.) was used as pre-emergence herbicide. Corn plants were not irrigated at this location because rainfall is normally considered adequate.

6.2.2 Sample Collection

Soil samples were collected between 0-10 cm from the soil surface from blocks 1 and 2 of the aforementioned research plots. Three biochar treatment levels were chosen. The 0, 2, and 4% treatment levels were chosen, as those are the levels most applicable to agricultural growers in similar climates to the experimental site. Four samples were taken from within each of the plots for a total of 24 samples and were stored at -20°C until extraction.

6.2.3 DNA Extraction

For each sample, DNA was extracted from 0.25 grams of soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's protocol (Appendix A). Extracted samples were stored at 4oC until processing.

6.2.4 DNA Amplification and Sequencing

Quality control, library preparation, amplification and sequencing were all completed by SeqMatic Laboratories, LLS (Fremont, CA).

Purified DNA concentrations from the 24 isolated samples were validated using Qubit Broad Range assay for DNA concentration determination. Illumina adapters and sample identifying barcodes were attached to the individual DNA samples and then amplified, targeting the V4 region (Youssef et al., 2009), by direct PCR using the primers 515F and 806rB following the Earth Microbiome 16S rRNA Amplification protocol (Caporaso et al., 2010, 2012).

515f: GTGCCAGCMGCCGCGGTAA (linker GT)

806rB: GGACTACNVGGGTWTCTAAT (linker CC)

Figure 19: Primer Sequences for V4 region of 16S Bacteria and Archaea rRNA

Samples were normalized and pooled into a single tube. The total DNA pool was gel isolated to purified and remove spurious bands from the library, then validated using an Agilent TapeStation DNA assay (Agilent Technologies, Santa Clara, CA). The pooled samples were then sequenced using a 150 base pair pair-ended read on the Illumina MiSeq Platform (Illumina, Inc., San Diego, CA). Resulting sequences were demultiplexed, barcode and primer sequences were trimmed and output into FASTQ format.

6.2.5 QIIME Workflow for Analysis of Sequenced Data

FASTQ files containing the sequencing data of each of the twenty-four samples supplied by SeqMatic (Fremont, CA) were processed through the open-source software, Quantitative Insights Into Microbial Ecology (QIIME). Pair-ended reads were analyzed for overlap and matching ends were combined for maximum sequence length using fast length adjustment for short reds (FLASH) (Magoc and Salzberg, 2011). Chimeric sequences were identified and removed from further identification in QIIME using ChimeraSlayer (Haas et al., 2011). Sample files were assembled into OTUs (picked at the 97% similarity threshold) and aligned with a set of reference sequences to assign taxonomy using the SILVA database (Quast et al., 2013). Analysis of alpha diversity was tested with Shannon and Simpson diversity indices as well as richness (number of observed OTUs). Alpha diversity and differences among taxa were analyzed by one-way ANOVA using the GLM Procedure in SAS 9.4 (SAS Institute Inc. Cary, NC). Means separated by Tukey's HSD at significance level of $p < 0.05$.

6.3 Results and Discussion

The sequences in this study are from the V4 region of 16S rRNA, this has been reported as a superior region to sequence for phylogenetic assignment and overall sample richness estimation (Youssef et al., 2009). This region contains approximately 250bp and sufficient coverage of the V4 region was obtained with a 150bp paired-end read. This allowed overlap of the forward and reverse sequences for FLASH (Magoc and Salzberg, 2011). Aligning merged reads allowed for greater taxonomic assignment using the ARB Silva database (Quast et al., 2013).

Average read length of the sequences used in this study was 253 base pairs long and sample counts ranged from 308156 to 718005 with a mean of 508394 reads and a total of 12201451. There was no statistical difference between extracted DNA sample concentrations or the number of sequences read due to biochar amendment as compared to the control. Of 12201451 total reads, only 3777 could not be classified to any phylum and the remaining reads were identified and placed into 16320 different taxonomic classifications.

6.3.1 Overall Sample Characteristics

The relative abundance of bacteria was examined at the phylum, class, and genus levels. Over all twenty-four soil samples, 51 (3 archaea) phyla, 183 (9 archaea) classes, and 1145 (21 archaea) genera were present. The most abundant phyla for all samples were the proteobacteria and acidobacteria, together comprising over 50% of the sequenced rRNA. Of the proteobacterial classes, alpha-, beta-, delta- and gammaproteobacteria were amongst the highest abundant classes with alphaproteobacteria being the most abundant

proteobacteria class, only topped by acidobacteria-6 in terms of overall class abundance in each plot. Other phyla of high abundance include the actinobacteria, chloroflexi, planctomycetes, verrucomicrobia, bacteriodetes, nitrospirae, gemmatimonadetes, crenarchaeota (archaea) and firmicutes.

The calculated alpha-diversity indices (Shannon and Simpson) showed no significant differences between biochar-amended soils and the control. The number of observed OTUs did increase from 7623 in the control soil to 7778 and 7657 in the 2% and 4% biochar amended soils, respectively, however, these differences were also not statistically significant. Using taxonomic assignments and looking closer at the bacterial community composition, significant differences between taxonomic groups at multiple levels are present.

Individual OTU differences were found at the phylum, class and genus taxonomic levels. Of the 51 phyla identified, 8 were significantly affected by the addition of biochar to the soil. The Fimicutes (Bacteria) and the Euryarchaeota (Archaea) were both negatively affected by increasing biochar amendment, whereas the bacterial phyla of Chlamydiae, Elusimicrobia, Verrucomicrobia, Armatimonadetes, TM6, and Kaza-3B-28 were all positively affected by the biochar amendment. TM6 and Kazan-3b-28 are considered candidate phyla by ARB-SILVA (Quast et al., 2013). The Proteobacteria, Acidobacteria and Actinobacteria were also positively affected by the addition of biochar to the research soil. These three phyla comprise over 60% of the overall abundance, however, differences were not statistically significant. Looking closer at these three phyla, six classes (two Acidobacteria, three Actinobacteria and one Proteobacteria class) within these three phyla increased significantly in the biochar treated soils and four classes decreased significantly (all Acidobacteria).

107

While factors affecting the microbial community are vast, pH has a significant documented effect on microbial activity and diversity (Fierer and Jackson, 2006; Rousk et al., 2010). The effects of pH are seen at the macroscale with changes in overall abundance and microbial respiration to the microscale, where individual bacterial taxa are influences by their environmental pH. The soil pH increased from 5.84 in the control treatment to 6.19 and 6.40 in the 2 and 4% of biochar treatment levels, respectively. This increase is significant statistically and these plots have maintained significant differences for the full length of this study. The range of soil pH values observed in this study fall within 0.6 points of each other and thus should not have a large impact on the overall bacterial abundance as was the case in this study. Specific taxa however, will respond individually to the shift in pH. The response of the Acidobacteria phyla as a whole was not significant, however six Acidobacteria classes responded significantly (four negative and two positive) to soil pH. This varied response has been previously documented as specific Acidobacteria classes tolerate a specific pH range (Jones et al., 2009). Many other bacteria taxonomic groups have not been assessed for their specific response to soil pH changes and more targeted study is necessary to evaluate this further.

6.3.2 Nitrogen Cycling Bacteria

Of the genera sequenced and identified, several are directly involved with the cycling of nitrogen in the soil. Three nitrifying genera, Nitrosovibrio, Nitrosomonas and Nitrosovibrio were present in all tested field plots. Nitrosovibrio and Nitrosomonas, (ammonium oxidizers) abundance both decreased (significantly and non-significantly, respectively) with increasing biochar amendment while the Nitrospira genus increased in both the 2 and 4% biochar-amended soils as compared to the control. While the Nitrospira

increase was not significantly significant, the percentage of Nitrospira is over ten times that of the other two nitrifying genera combined at each biochar treatment level. Due to the greater abundance of Nitrospira overall, biochar induced increases may have a greater effect on nitrification that Nitrosomonas and Nitrosovibrio. A recent study also reported an increased Nitrospira abundance in biochar amended rice paddy topsoil in two of the three sites studies in South China (Chen et al., 2015). Chen et al. also found increases in two other nitrifying genera, Nitrosococcus and Nitrosopira, which were not identified in this soil. The reduction of ammonia oxidizer abundance with increasing biochar amendment suggests interference with the oxidative process or the unavailability of ammonium potentially due to greater adsorption to the biochar surface functional groups. The increase in nitrite-oxidizing Nitrospira may suggest sufficient increased nitrite availability or an increased in Nitrospira activity due to an increase in soil pH.

Nitrogen-fixing bacterial genera, Bradyrhizobium, Frankia, and Rhizobium were also assessed for their relative abundance across biochar treatment levels. All three of these nitrogen-fixing genera had greater abundance in the biochar-amended soils as compared to the control. Bradyrhizobium was by far the most abundant and was also the only genus to increase significantly in biochar-amended soils. Bradyrhizobium over 21% from the control level of 1.190% to 1.446% in the 2% biochar treatment level, and while the Rhizobium increase was not significant, the mean abundance increased 300% from 0.017% to 0.68% in the 2% biochar treatment level. An increase in Bradyrhizobium abundance in biochar-amended topsoils was also reported by Chen et al. (2015).

Equally important in regulating soil nitrogen processes are the denitrifying bacterial genera. In the soils studied here, three key genera were identified. Paracoccus, Geobacter and Anaeromyxobacter. In total, these bacteria were less abundant than both the nitrifying

and nitrogen-fixing genera identified and discussed previously. Over half of the species that fall within the Paracoccus genus are denitrifying bacteria and many are commonly found in soil (Kelly et al., 2006). The abundance of this genus decreased drastically in both biochar-soil treatments as compared to the control. In contrast, the Geobacter genus abundance almost doubled in the soils amended with 2% biochar. The geobacter genus is composed of species with diverse metabolic pathways, but typically are found in anaerobic conditions and several species (including the type-species G. *metallireducens*) reduce Fe(III) along with oxidation of organic carbon-compounds (Coates et al., 1996). The increase in this genus in the 2% biochar treatment level may indicate an increase in micro-anaerobic sites within the biochar pores. However, the overall decline in abundance quantified in the 4% treatment level is much lower than the control indicating that there may be other factors such as Fe(III) availability or soil environmental conditions such as the increase in soil pH affecting this genus.

The recalcitrance of biochar is soil has been touted as one of its benefits as a soil amendment. It has been reported and now generally accepted that biochar carbon is not mineralizable by soil microbes (Thies and Rillig, 2009). However, small percentages of carbon may be labile and available, and along with changes to the nutrient availability and pH, studies have shown increases in soil microbial communities (Warnock et al., 2007; Steiner et al., 2008; Liang et al., 2010; Zimmerman et al., 2010, 2011). No major differences in bacterial genera Chitinophaga, Flavobacteirum, and Cellvibrio, were detected. These bacteria are capable of degrading carbon substrates such as chitin and other biopolymers and have previously been reported to increase in abundance with biochar application to soils (Kolton et al., 2011).

6.4 Conclusion

Overall, the changes in the microbial communities with the addition of biochar were modest, potentially indicating an overall temporal-stability of diverse microbial communities in soil after biochar amendment. While individual taxa were significantly affected, there was no overall significant difference in total abundance, observed OTUs or alpha diversity indices. It is suggested, however, that a 4% (w/w) biochar amendment may have limited benefits, as alpha diversity, whole tree phylogenetic diversity and observed OTUs all declined when compared to a 2% (w/w) biochar amendment. An application rate of 2% (81 t ha^{-1}) biochar to the temperate agricultural soils studied here had a priming effect on species diversity as compared to untreated soil. Coupled with the small agronomic benefit reported earlier at this treatment level, this study recommends an application rate of no more than 2% using the biochar and soil presented here.

Table 8: Concentration of bacterial DNA and number of reads from Illumina MiSeq Sequencing

Plot ID	Biochar	Tech. Rep.	Conc. (ng uL^{-1})	Number of Reads
C1	0%	1	45.47	715,115
		2	43.41	586,045
		3	40.28	670,616
		4	62.72	983,858
C2	0%	1	33.36	582,316
		2	53.08	578,611
		3	39.93	561,130
		4	51.49	1,032,864
B1	2%	1	42.83	443,491
		2	68.67	698,599
		3	37.29	802,431
		4	38.79	1,041,100
E2	2%	1	53.46	784,575
		2	50.82	514,914
		3	50.24	834,587
		4	35.75	1,065,117
B2	4%	1	42.19	836,677
		2	40.00	766,752
		3	46.28	670,465
		4	39.14	960,239
D1	4%	1	49.42	494,642
		2	42.65	491,035
		3	41.25	834,390
		4	28.62	997,075
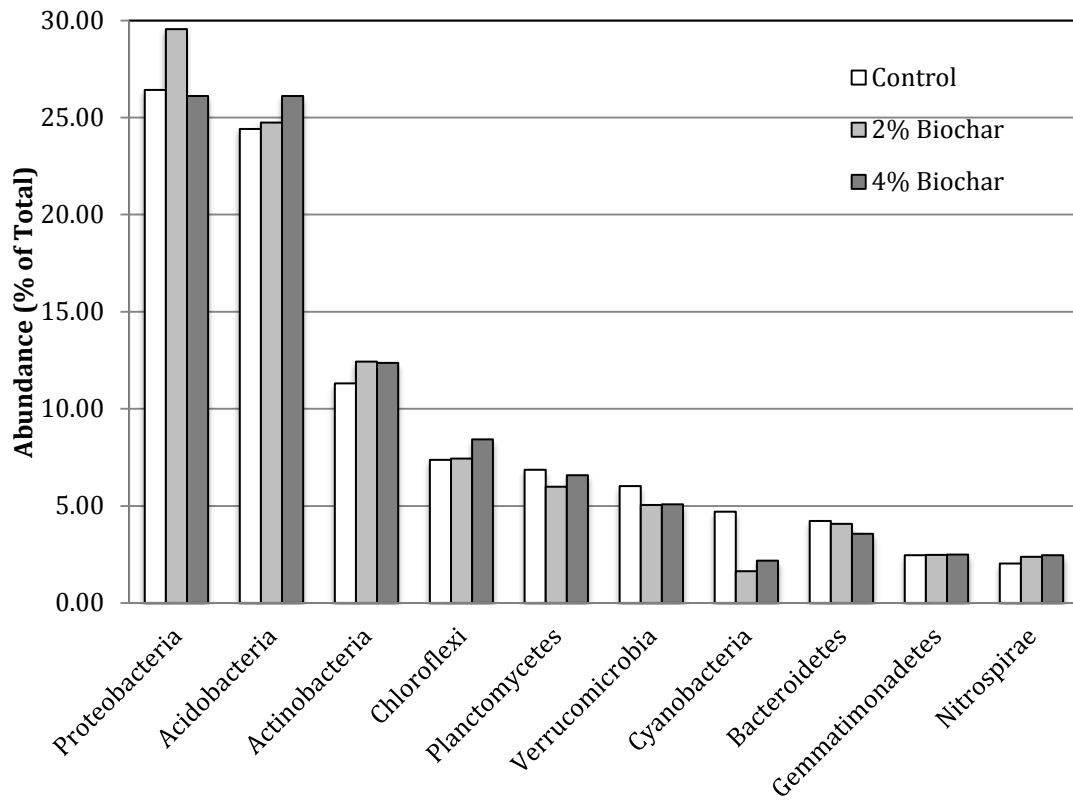
Figure 20: Phylum Abundance of the ten most abundant bacterial phyla.

Treatment means presented as percentage of total sequenced DNA

per treatment level.

Table 9: Significant differences in taxa at the Phylum level

Phylum	Biochar Treatment			P > f
	0%	2%	4%	
TM6*	**0.0659**	0.0553	0.0342	0.0419
Euryarchaeota (A)	0.1526	0.1661	**0.2528**	0.0358
Chlamydiae	**0.0155**	0.0101	0.0069	0.0344
Elusimicrobia	**0.1446**	0.1284	0.1176	0.0274
Verrucomicrobia	**6.0238**	5.0411	5.0832	0.0214
Armatimonadetes	**0.5640**	0.4657	0.4734	0.0199
Firmicutes	0.9763	1.2654	**1.3618**	0.0178
Kazan-3B-28*	**0.0016**	0.0003	0.0000	0.0049

(A) Archaea

* Candidate phyla

Table 10: Significant differences in taxa at the class level

Phylum	Class	Biochar Treatment			P > f
		0%	2%	4%	
Euryarchaeota (A)	Methanobacteria	**0.0083**	0.0096	0.0178	0.0056
Crenarchaeota (A)	MBGA	**0.0063**	0.0036	0.0012	0.0133
Acidobacteria	Acidobacteriia	**0.6097**	0.4491	0.3133	0.0009
Acidobacteria	Holophagae	**0.0040**	0.0022	0.0006	0.0194
Acidobacteria	Solibacteres	**1.0164**	0.9419	0.7879	0.0022
Acidobacteria	DA052	**0.1845**	0.1303	0.0946	0.0168
Armatimonadetes	Chthonomonadetes	**0.1992**	0.1420	0.1332	0.0067
Chlamydiae	Chlamydiia	**0.0155**	0.0101	0.0069	0.0344
Chloroflexi	Ktedonobacteria	**0.0387**	0.0304	0.0270	0.0112
Chloroflexi	C0119*	**0.0605**	0.0451	0.0366	0.0339
Cyanobacteria	4C0d-2*	**0.1770**	0.1603	0.1195	0.0051
Elusimicrobia	Elusimicrobia	**0.1420**	0.1254	0.1152	0.0240
Firmicutes	Clostridia	**0.2874**	0.2865	0.2252	0.0298
Kazan-3B-28*	(U)	**0.0016**	0.0003	0.0000	0.0049
TM6*	SJA-4*	**0.0648**	0.0532	0.0329	0.0404
Verrucomicrobia	Spartobacteria	**4.2830**	3.2751	3.4125	0.0143
Proteobacteria	Deltaproteobacteria	4.0918	**5.0858**	4.3498	0.0003
Chlorobi	BSV26*	0.0096	**0.0213**	0.0177	0.0056
Planctomycetes	(U)	0.0296	**0.0379**	0.0290	0.0221
Acidobacteria	RB25*	0.0399	**0.0644**	0.0602	0.0122
Acidobacteria	S035*	0.1088	0.1538	**0.1859**	0.0016

Actinobacteria	Acidimicrobiia	1.8797	2.3473	**2.4668**	0.0181
Actinobacteria	Nitriliruptoria	0.1825	0.2573	**0.3134**	0.0071
Actinobacteria	MB-A2-108*	0.0000	0.0005	**0.0015**	0.0172
Chloroflexi	Anaerolineae	1.6577	2.1425	**2.5876**	0.0012
Firmicutes	Bacilli	0.6850	0.9737	**1.1338**	0.0037
Gemmatimonadetes	Gemm-2	0.0213	0.0368	**0.0436**	0.0036
Planctomycetes	028H05-P-BN-P5*	0.0081	0.0255	**0.0340**	0.0020
WS2*	Kazan-3B-09*	0.0000	0.0003	**0.0006**	0.0450
GN02*	BD1-5*	0.0002	0.0001	**0.0009**	0.0301
Tenericutes	RF3*	0.0005	0.0004	**0.0010**	0.0281

(A) Archaea

(U) Unnamed Class

*Candidate taxa

Table 11: Significant differences in taxa at the family level

Family	Biochar Treatment			P>F
	0%	2%	4%	
Acetobacteraceae	**0.2078**	0.1439	0.0992	0.0002
Actinomycetales (U)	**0.3520**	0.3286	0.2480	0.0358
Alcaligenaceae	**0.0380**	0.0239	0.0251	0.0010
Aurantimonadaceae	**0.0171**	0.0037	0.0028	0.0381
Chthoniobacteraceae	**4.2824**	3.2748	3.4120	0.0143
Chthonomonadaceae	**0.1079**	0.0685	0.0677	0.0032
Clostridiaceae	**0.1220**	0.0942	0.0682	0.0311
Coxiellaceae	**0.1354**	0.0965	0.0763	0.0010
Ellin329 (U)	**0.4138**	0.3814	0.3019	0.0022
Ellin6513 (U)	**0.1845**	0.1303	0.0946	0.0168
Ellin6537 (U)	**0.0295**	0.0131	0.0099	<0.0001
FAC88 (U)	**0.0554**	0.0523	0.0402	0.0253
Holophagaceae	**0.0040**	0.0022	0.0006	0.0194
Isosphaeraceae	**0.4863**	0.3568	0.3422	0.0036
Koribacteraceae	**0.5976**	0.4359	0.3074	0.0009
MLE1-12 (U)	**0.1474**	0.1360	0.0995	0.0062
Nitrosomonadaceae	**0.0499**	0.0322	0.0250	0.0289
Pedosphaeraceae	**0.0208**	0.0177	0.0134	0.0463
Rhodocyclaceae	**0.0337**	0.0201	0.0129	0.0030
SAGMA-X (A)	**0.0770**	0.0106	0.0037	<0.0001
Solibacteraceae	**0.5040**	0.4502	0.3687	0.0035
Solibacterales (U)	**0.4937**	0.4587	0.3886	0.0078
Thermogemmatisporaceae	**0.0249**	0.0111	0.0130	0.0190
Bdellovibrionaceae	0.0954	**0.1200**	0.1049	0.0296

Bradyrhizobiaceae	1.3327	**1.6309**	1.4819	0.0243
Cystobacteraceae	0.1494	**0.4096**	0.1794	0.0004
Geobacteraceae	0.0315	**0.0622**	0.0139	0.0170
Hyphomicrobiaceae	3.4614	**4.3771**	4.0826	0.0109
OM27	0.0576	**0.0680**	0.0505	0.0278
Paenibacillaceae	0.0863	**0.1167**	0.1049	0.0454
Pedosphaerales (U)	0.3116	**0.3261**	0.2625	0.0122
Rhodospirillaceae	1.3688	**1.6872**	1.3979	0.0064
Solirubrobacteraceae	0.3642	**0.4996**	0.4230	0.0349
0319-7L14 (U)	0.1005	0.1368	**0.1508**	0.0421
Acidimicrobiales (U)	0.6582	0.7831	**0.9219**	0.0065
Anaerolinaceae	0.0012	0.0029	**0.0058**	0.0031
Ardenscatenaceae	0.0028	0.0068	**0.0167**	0.0032
Bacillaceae	0.1190	0.1472	**0.1731**	0.0256
Beijerinckiaceae	0.0175	0.0288	**0.0328**	0.0350
Caldilineaceae	0.0722	0.0856	**0.1360**	0.0067
Cenarchaeaceae (A)	0.1765	0.2344	**0.3948**	0.0155
Chromatiales (U)	0.0066	0.0371	**0.0384**	0.0022
Entotheonellaceae	0.0380	0.0598	**0.0632**	0.0271
Euzebyaceae	0.0000	0.0005	**0.0015**	0.0172
H39 (U)	0.5632	0.7647	**0.7594**	0.0362
Iamiaceae	0.0551	0.1665	**0.2413**	0.0020
Mariprofundaceae	0.0156	0.0231	**0.0232**	0.0496
mb2424	0.3083	0.4404	**0.7034**	0.0002
Methanobacteriaceae (A)	0.0083	0.0096	**0.0178**	0.0056
OPB35	0.0367	0.0708	**0.0785**	0.0300
Pirellulaceae	0.8951	0.8841	**1.0859**	0.0344
PK29 (U)	0.1175	0.1383	**0.1810**	0.0025
Planococcaceae	0.1950	0.4327	**0.5995**	<0.0001

Rhizobiales (U)	0.4132	0.4881	**0.4997**	0.0389
Solirubrobacterales (U)	0.5121	0.6209	**0.7211**	0.0076
Streptomycetaceae	0.2064	0.2593	**0.3150**	0.0065

(A) Archaea

(U) Unnamed family within listed class

Table 12: 36-month average pH of biochar treatments

Biochar Rate	pH
0%	5.84
2%	6.19
4%	6.40

Table 13: Diversity Indices of sequenced rRNA in biochar amended field soils

Diversity Index	Biochar Treatment		
	0%	2%	4%
H'	10.205	10.296	10.281
λ	0.997	0.998	0.998
Observed OTUs	7623	7778	7657

H' = Shannon Diversity Index

λ = Simpson Index

OTUs determined after chimera removal

Table 14: Abundance, as percentage per sample, of select N-processing bacteria

Genus	N-Process	Biochar Treatment			P > f
		0%	2%	4%	
Nitrosovibrio	Nitrifying	0.0429	0.0243	0.0172	0.006
Nitrosomonas	Nitrifying	0.0013	0.0004	0.0006	ns
Nitrospira	Nitrifying	0.4609	0.5605	0.5140	ns
Bradyrhizobium	Fixing	1.1895	1.4455	1.2730	0.038
Frankia	Fixing	0.0037	0.0042	0.0046	ns
Rhizobium	Fixing	0.0169	0.0683	0.0352	ns
Paracoccus	Denitrifying	0.0013	0.0002	0.0001	0.072
Geobacter	Denitrifying	0.0315	0.0622	0.0139	0.017

APPENDIX

MOBIO POWERSOIL DNA EXTRACTION EXPERIENCED USER PROTOCOL

1. To the PowerBead Tubes provided, add 0.25 grams of soil sample.

2. Gently vortex to mix.

3. Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.

4. Add 60 μl of Solution C1 and invert several times or vortex briefly.

5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.

6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature. CAUTION: Be sure not to exceed 10,000 x g or tubes may break.

7. Transfer the supernatant to a clean 2 ml Collection Tube (provided). Note: Expect between 400 to 500 μl of supernatant. Supernatant may still contain soil particles.

8. Add 250 μl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

10. Avoiding the pellet, transfer up to, but no more than, 600 μl of supernatant to a clean 2 ml Collection Tube (provided).

11. Add 200 μl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.

12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

13. Avoiding the pellet, transfer up to, but no more than, 750 μl of supernatant into a clean 2 ml Collection Tube (provided).

14. Shake to mix Solution C4 before use. Add 1200 μl of Solution C4 to the supernatant and vortex for 5 seconds.

15. Load approximately 675 μl onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 675 μl of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Note: A total of three loads for each sample processed are required.

16. Add 500 μl of Solution C5 and centrifuge at for 30 seconds at 10,000 x g.

17. Discard the flow through.

18. Centrifuge again at room temperature for 1 minute at 10,000 x g.

19. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.

20. Add 100 μl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

21. Centrifuge at room temperature for 30 seconds at 10,000 x g.

22. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required. We recommend storing DNA frozen (-20° to -80°C). Solution C6 contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

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