BIOCHAR FOR THE BRAZILIAN CERRADO: CONTRIBUTIONS TO SOIL QUALITY AND PLANT GROWTH

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES
(Resource Management and Environmental Studies)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

August 2017

Abstract

Arenosols (sandy soils) in the Cerrado region of Mato Grosso, Brazil are increasingly used for maize production. These soils are typically nutrient poor with low soil water retention. Since biochar has been shown to improve both nutrient and water retention, this thesis aimed to evaluate biochar effect on physical and chemical properties of a Cerrado Arenosol using four biomass wastes (cotton husks, swine manure, eucalyptus sawmill residue, and sugarcane filtercake) pyrolyzed at three temperatures (400°, 500°, 600°C). These biomass wastes were chosen based on their prevalence in the state of Mato Grosso and their environmental impact. Three greenhouse experiments were carried out with the following objectives: 1) to assess the effects of biochar feedstock type and temperature of pyrolysis on soil water retention; 2) to examine the effect of different biochars on soil nutrients and maize growth applied to soil at different rates, and 3) to observe how different biochar feedstocks and temperatures of pyrolysis affect DOC and NO_3^- leaching from a Cerrado Arenosol. All the biochars showed potential to reduce water drainage in the soil compared to the control (no biochar). At application rates 1-4% w/w, filtercake biochar led to the highest mean biomass compared to the other biochars. Eucalyptus biochar did not contribute much to soil fertility, but filtercake biochar led to high soil nutrient concentrations, e.g. Ca, Fe, Mn. Although swine manure biochar was rich in nutrients, low plant biomass in the cotton and swine manure biochar treatments was likely due to higher pH, salinity, and/or excessive water retention. Lastly, DOC and NO_3^- concentrations were low in leachate from soils with filtercake and eucalyptus biochars, and high in leachate from soils with cotton and swine manure biochars. This thesis provides an outlook of the agronomic potential of these biochars. Further analyses, such as their effect on soil biological properties, are required to develop well-rounded biochar-soil management practices for Cerrado Arenosols.

Lay Summary

Sandy soils in the Cerrado region of Mato Grosso, Brazil are increasingly used for maize production. These soils are typically nutrient poor with low soil water retention. Since biochar (charcoal made of organic wastes) has been shown to improve both soil nutrient and water retention, this thesis aimed to evaluate biochar effect on physical and chemical properties of a Cerrado sandy soil when mixing in biochars made from different agricultural waste materials heated at different temperatures. The waste materials used were chosen based on their prevalence in the state of Mato Grosso and their environmental impact. Through greenhouse experiments and laboratory analyses, this thesis provides an outlook of the agronomic potential of these biochars, which varied depending on the waste material and amount added to the soil. Additional analyses, such as their effect on soil biological activities, will provide a well-rounded image of their contributions to a Cerrado sandy soil.

Preface

Chapters 1 and 5 are the sole work of the author based on a review of the literature. All writing of these and other chapters was the sole responsibility of the author.

A version of **Chapter 2** has been published (Speratti, A.B., Johnson, M.S., Martins Sousa, H., Nunes Torres, G., Guimarães Couto, E., 2017. Impact of different agricultural waste biochars on maize biomass and soil water content in a Brazilian Cerrado Arenosol. *Agronomy*, 7, 49) and **Chapter 4** has been prepared as a manuscript to submit to a scientific journal. Both chapters are based on a greenhouse experiment carried out at the Universidade Federal de Mato Grosso (UFMT), Cuiabá, Brazil from November to December 2014 by the author and Heiriane Martins Sousa. The author was responsible for laboratory and data analyses. Gilmar Nunes Torres assisted with the collection of intact soil cores from pots and measurement of soil water retention in the laboratory, as well as carried out the particle size analysis described in **Chapter 2**. Fluorescence spectroscopy analysis described in **Chapter 4** was performed with the assistance of Dr. Higo J. Dalmagro. Dr. Mark S. Johnson contributed to experimental design, data analysis, and critical revision of the manuscripts, and Dr. Eduardo Guimarães Couto contributed to experimental design. We thank Edmar de Queiroz, André Espinoza, Andrei Oliveira, and Vandir Soares for their assistance in the greenhouse. Special thanks to the following UFMT professors: Francisco Lobo and Carmen E.R. Ortiz for laboratory space, Dr. Ricardo S. Amorim for assistance calculating the water curves, and Dr. Ailton J. Terezo for SEM analysis of the biochars. We also thank Haiyan Wang at UBC for biochar porosity analysis and Afonso da Silva from Grupo Bom Futuro, Campo Verde.

Chapter 3 was prepared as a manuscript to submit to a scientific journal, based on a greenhouse experiment carried out at the UFMT, Cuiabá, Brazil from February to March 2015 by the author and Heiriane Martins Sousa. The author was responsible for laboratory and data analyses. Plant physiological data collection was performed with the assistance of Dr. Higo J. Dalmagro. Dr. Mark S. Johnson contributed to experimental design and critical revision of the manuscript, and Dr. Eduardo Guimarães Couto contributed to experimental design. We thank the aforementioned in **Chapters 2 and 4** for their continued assistance in the greenhouse and laboratory, along with Giselle Ferreira, and Christie Hilleshein Cardoso and Flavia Louyze for CHN analysis.

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List of Abbreviations

AWC – available water content

BIX – biological index

CEC – cation exchange capacity

DOC – dissolved organic carbon

DOM – dissolved organic matter

E – transpiration rate

EC – electrical conductivity

EEMs – excitation-emission matrices

E_{plant} – plant aboveground transpiration

FI – fluorescence index

GHG – greenhouse gases

g_{plant} – plant stomatal conductivity

g_s – stomatal conductance

HIX – humification index

K – hydraulic conductivity

K_s – saturated hydraulic conductivity

NO_3^- – nitrate

NUE_{Prod} – leaf nitrogen use efficiency

PARAFAC – parallel factor analysis

PCA – principal component analysis

P_n – Net photosynthetic rate

$PNUE_{plant}$ – photosynthetic nitrogen use efficiency

P_{plant} – plant photosynthesis

R_d – dark respiration rate

R_{plant} – aboveground plant respiration

SOC – soil organic carbon

SOM – soil organic matter

VWC – volumetric water content

WHC – water-holding capacity

WUE_{Prod} – water use efficiency of productivity

WUE_s – intrinsic water use efficiency

WUE_t – water use efficiency of photosynthesis

Glossary

Aromaticity – describes the stability of organic compounds

Arenosol – deep, very sandy soils derived from the weathering of quartz-rich materials

Cerrado – Brazil’s second largest biome characterized by savanna, gallery forests, and grassland vegetation

Electrical conductivity – measures the soil’s ability to transport charged solutes, suggesting nutrient levels and crop yield potential

Ferralsol – intensely weathered, well-drained soils characterized by accumulation of iron and aluminum oxides

Humification – binding of more resistant organic components to soil minerals, becoming a stabilized pool of SOM (humus)

Hydraulic conductivity – ease of water movement through the soil

Mineralization – microbially-driven transformation of organic elements into inorganic compounds available for plant and microorganism uptake

Leachate – liquid that has drained from soil, containing nutrients and DOM

Acknowledgements

This work would not have been possible without the assistance of many students and professors in the Programa de Pós-Graduação em Agricultura Tropical, Faculdade de Agronomia e Zootecnia at the UFMT. I particularly appreciate the support and friendship of Heiriane Martins Sousa during my time in Cuiabá. Not only was my research able to progress more smoothly thanks to her help and familiarity with the UFMT, but my experience would have been less enjoyable without our daily *almoços* at the RU and overall emotional support. I was also fortunate enough to coincide with Dr. Luisa Vega's post-doc at the UFMT and have had a fellow Spanish-speaker and friend. Special thanks to our collaborator, Dr. Eduardo Guimarães Couto, as well as Ms. Suzana Souza dos Santos, who was essential in coordinating logistics. I thank UFMT professors Drs. Francisco Lobo, Oscarlina Weber, Fernando Scaramuzza, and Ricardo Amorim for access to their laboratories. I thank Higo Dalmagro for his assistance with the Aqualog and all his advice, and Gilmar Nunes Torres and the other students of the Programa de Pós-Graduação em Agricultura Tropical for their unfailing upbeat attitude and support of one another, including myself whenever I needed help.

My time in Cuiabá, of course, was possible thanks to faculty, staff and students of the Institute for Resources, Environment and Sustainability at UBC. I especially thank my supervisor Dr. Mark S. Johnson for his guidance and fellow Ecohydro lab students for their support, particularly Mike Lathullière, my UBC companion in Cuiabá. My committee members, Drs. Maja Krzic and Sean Smukler, provided valuable input.

This work was financially supported by student awards from the Canadian Natural Sciences and Engineering Research Council (NSERC) Post-Graduate Scholarship-Doctoral Award, an NSERC-Create TerreWEB Scholarship, and a UBC Four Year Fellowship. Other support included the Belmont Forum and the G8 Research Councils Freshwater Security Grant (G8PJ-437376- 2012) through NSERC and a research grant from the Brazilian National Council for Scientific and Technological Development (CNPq).

Lastly, a big thank you to my family and friends for all their love and encouragement, and in particular to Luis, for his incredible patience, support and unwavering confidence in me during the most trying times.

Dedicado a la memoria de
mi abuelo Nenito
(27/4/1921 – 8/7/2017)

Chapter 1: Introduction

1.1 The Cerrado biome

The Cerrado, located south of the Amazon and covering central Brazil (Figure 1.1), is the second largest biome in Brazil, after the Amazon rainforest (Buol, 2009). Its natural vegetation consists of savanna, gallery forests, and grassland, with fire-adapted and dependent vegetation. Characterized by wet and dry seasons, it receives between 1200 to 2000 mm of rain annually (Batlle-Bayer et al., 2010). Soils are predominantly Ferralsols (FAO classification), deep, intensely weathered, well-drained soils characterized by accumulation of iron (Fe) and aluminum (Al) oxides, low pH, and low nutrient (particularly phosphorus, P) content (Batlle-Bayer et al., 2010). Ferralsols cover 46% of the Cerrado, but other major soil groups in the region include Acrisols (15%) and Arenosols (15%) (EMBRAPA, 1999).

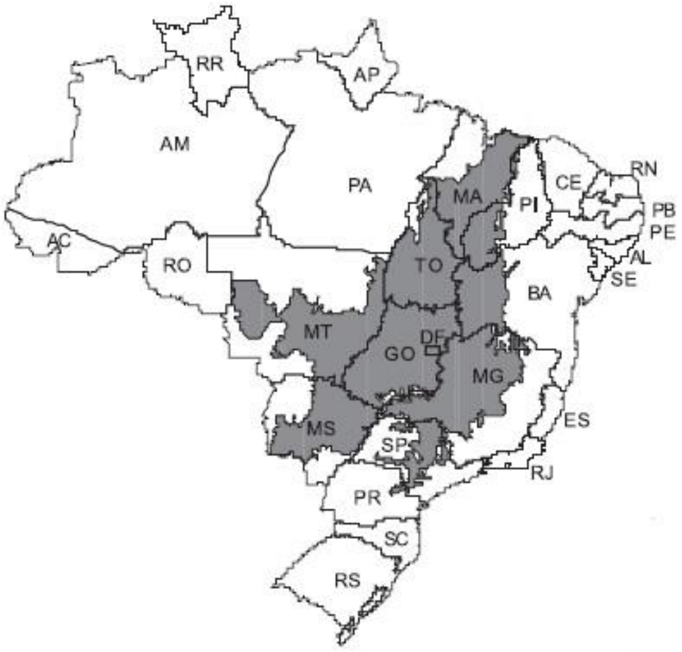

Figure 1.1. Map of Brazil showing area under Cerrado (shaded) (from Sano et al., 2008). MT is the state of Mato Grosso

Arenosols are of particular interest in this region since their use as cultivated soils, mainly for growing maize, is increasing. Arenosols are sandy soils derived from the weathering of quartz-rich materials, hence the name of Quartzipsamments in the USDA soil classification and Neossolos Quartzarênicos in the Brazilian soil classification. They are characterized by an A horizon directly over a large C horizon, with a loamy sand or coarser texture to a depth of 100 cm from the surface. The sand and silt fractions are dominated by quartz and feldspars, while the clay fraction depends on the parent material; some clay minerals include vermiculites, chlorites, and cemented kaolin. As they are mainly structureless, non-plastic, and non-sticky with high macroporosity (total porosity between 36 and 46%), Arenosols drain rapidly and have low water-holding capacity (WHC). Organic material in these soils is usually less than 1% and their cation exchange capacity (CEC) is low below the top 20 cm (ISRIC, 2015).

Due to its nutrient-poor and acidic soils, such as the Arenosols, for a long time the Brazilian Cerrado region was not considered of agricultural value (Buol, 2009). In the 1970s, the Brazilian government began studies which showed that through technical implementations, the region's productivity could be increased. Since then, with the use of fertilizers, liming, and introduction of exotic grasses, such as *Bracchiaria* sp, 50% of the Cerrado's natural vegetation has been transformed into pasture and agricultural croplands (Arantes et al., 2016). Agriculture in the region is predominantly comprised of double-crop rotation, mainly soybean production during the wet season (October to April) and sorghum, maize, or millet production during the dry season (May to September) (Lopes, 1996). Most agricultural crops, however, are not adapted to Cerrado soils, their rooting system limited to the surface where nutrient input is highest (Lopes, 1996). Nutrient levels in Cerrado soils have been observed to decrease with loss of soil organic matter (SOM) due to land-use conversion or improper management practices (da Silva et al., 2004). Common agricultural management practices in the Cerrado region include liming to increase pH, building up available P, and managing organic matter (OM) inputs (Lopes, 1996).

Building up nutrient levels in the soil is not the only concern for farmers in the Cerrado. The dry season, short droughts and high evapotranspiration rates during the rainy season, as well as the soil's naturally low WHC pose challenges for pasture grasses and agricultural crops. The native vegetation contributes to the Cerrado's hydrology through its adaptation to the rainfall patterns of the region and its deep roots. Removal of this vegetation for land-use conversion can thus have consequences on the region's freshwater stability (Oliveira et al., 2005). Conversion to pasture and agriculture can lead to lower carbon (C) stocks, reduced evapotranspiration, greater greenhouse gas emissions, and increased heat flux (Arantes et al., 2016). Regional climate models of the Cerrado for the year 2100 based on the IPCC A2 scenario (IPCC, 2000) have predicted temperature increases from 2°C to 4°C in the most severe scenarios, and precipitation decreases of 20 to 50% the current values (Bustamante et al., 2012). For an agricultural region dependent exclusively on rain, this could have significant implications on the livelihoods and economy of the region. With climate change it is necessary to adopt sustainable soil management practices that take into account crop limitations and changing hydrology to maximize production and prevent further soil degradation.

Some sustainable soil management practices that contribute to reducing greenhouse gas emissions from agriculture include conservation agriculture, improved maintenance of pastures, and new forms of integrated production such as livestock-crop integrated systems (Galford et al., 2013). Besides causing C losses as carbon dioxide (CO_2) through removal of plant biomass and increased soil respiration, land-use management can also cause C losses through above and belowground water flow (Cronan et al., 1992). Soil organic carbon (SOC) fractions such as dissolved organic C (DOC) and black C (e.g. from biochar) play an important role in the C cycle and the stability of SOC (Jiménez and Lal, 2006; Lehmann et al., 2006), but examination of the controls on their dynamics and fluxes, particularly in tropical soils, is limited. According to a meta-analysis of tropical crop yields vs. temperate crop yields, biochar can be particularly useful in improving crop yields in tropical acidic, nutrient-poor soils, which is significant considering over 50% of the world's potential arable soils are acidic (Jeffery et al., 2017). Within the setting of climate change and agricultural production in the Brazilian Cerrado and bordering Amazon region, this chapter describes the processes of decomposition and stabilization of SOM, followed by an examination of the stability of biochar and DOC dynamics.

1.2 SOM conservation

A way to improve resilience to regional climate change is by protecting SOM, which is the basis for C storage in soils (Batjes, 1996). Land-use conversion is known to cause degradation of natural ecosystems, including of soils through SOM loss, and has been considered the second highest source of C emissions after fossil fuel burning (Batlle-Bayer et al., 2010; Watson et al., 2000). SOM can be maintained and built up by implementing so-called recommended management practices (RMPs) that contribute to improved soil quality and thus greater crop productivity, as well as mitigate climate change by increasing C sequestration in the soil (Batlle-Bayer et al., 2010).

1.2.1 SOM sources and decomposition

To be able to maintain and/or build up SOM in the soil, it is necessary to understand what it consists of and how it becomes part of the soil. SOM is composed of a variety of pools including above and below-ground plant residues (considered primary resources), soil animal and microorganism residues (considered secondary resources), dissolved organic matter (DOM), root exudates, and humic compounds (Zech et al., 1997). Black carbon, burned biomass leftover from fire, also contributes to SOC (Czimczik and Masiello, 2007). A large proportion of SOM comes from plants. Plant residues (including leaves, fallen trees, branches, and dead roots), shed animal body parts, dead animals, animal excretions, and all secretions make up the litter. The litter is repeatedly digested and excreted, broken down further and further until it is unrecognizable as the original material (Adl, 2003).

Decomposition occurs in stages, from the most easily degradable fractions consumed first to the most resistant last. These fractions are 1) lipids, proteins, and nucleic acids; 2) carbohydrates, which include starch, cellulose, and hemicellulose; and 3) lignin. The organisms responsible for the degradation of organic material are mainly microorganisms (bacteria and fungi). Microorganisms decompose organic material through their metabolism, obtaining energy and C sources (Swift et al., 1979), while macroinvertebrates, such as arthropods, earthworms, and gastropods, contribute through fragmentation and comminution of the litter (Coleman et al., 1988). The process of decomposition leads to two sub-processes: mineralization and humification.

1.2.2 Mineralization and humification

The quantity and chemical composition of the resources contributing to the SOM control its dynamics. During decomposition, the more labile SOM pools are rapidly mineralized into available nutrients. This is called mineralization, defined as the microbially-driven transformation of organic elements, e.g. C, P, nitrogen (N), and sulfur (S), into inorganic compounds available for plant and microorganism uptake, such as ammonium (NH_4^+), nitrate (NO_3^-), and sulfate (SO_4^{2-}), or lost to the atmosphere (e.g. CO_2, methane (CH_4)). As decomposition continues, mineralization slows down and less labile SOM components are stabilized through humification (Zech et al., 1997).

Humification allows SOM levels to remain stable (Jiménez and Lal, 2006). As the more labile components of SOM are mineralized, the more resistant components, such as lignin, remain. The resistant components bind to soil minerals, becoming a stabilized pool of SOM often called humus. Humus remains stable through both chemical and physical properties. Chemical fractions of humus include humic and fulvic acids and polysaccharides (Martin and Haider, 1971). Humic and fulvic acid molecules consist of complex polymers of phenolic units and can make up 50 to 80% of the humus, while polysaccharides consist of complex polymers of sugar units and can make up 10 to 30% of humus (Martin and Haider, 1971). Due to the complex aromatic structure of lignin (Crawford, 1981), few microorganisms are capable of decomposing it, although white rot fungi (*Basidiomycetes* sp.) have been found to be active lignin degraders (Bumpus, 1993).

Besides its chemical structure, humus can also be protected from decomposition by the physical structure of the soil. Clay particles can protect SOM by adsorbing and bonding through polyvalent cation bridges, forming tightly bound organo-mineral complexes within clay migroaggregates that prevent microogranisms' access to the OM within (Edwards and Bremner, 1967). For this reason, OM microaggregates with clay particles are more stable than aggregates with sand or silt particles (Edwards and Bremner, 1967). In addition, soils with high clay content contain higher porosity than coarse-textured soils. Smaller pores (<0.2 µm) in clay soils are less accessible to microorganisms and can thus protect OM from decomposition, as well as protect bacteria from predation by protozoa and nematodes. Reduced bacterial predation can lead to lower N mineralisation rates and OM with lower C:N ratio (Hassink et al., 1993). The physical protection of SOM by the soil structure is hence significant. In fact, the complex aromatic structure of lignin is ultimately broken down by fungi and remains less time in the soil compared to physically protected SOM (Grandy and Neff, 2008). Therefore, both the physical stability and chemical composition of SOM should be considered when examining its structure and formation.

1.3 Stabilization of SOM in tropical regions

SOM levels and SOC storage vary with external factors such as soil texture, climate, and land management. To complicate matters, SOC pools have considerably different decomposition rates which are affected by the aforementioned external factors (Craswell and Lefroy, 2001). Although their dynamics can differ, the fundamental processes of decomposition do not vary between tropical and temperate regions (Jenkinson, 1988). Soil texture and mineralogy, climate, and land-use conversion are important factors that also affect decomposition in tropical soils.

1.3.1 Soil texture and mineralogy

As mentioned, clays play a significant role in the physical stabilization of SOM, mainly in the formation of organo-mineral aggregates. Soil mineralogy also contributes to a soil’s ability to retain SOC, and may be of greater importance in tropical soils than in temperate soils (Nayak et al., 1990). The high clay content of Amazonian Ferralsols and Acrisols plays an important role in the stabilization of SOM because of its interaction with polyvalent cations, in particular Fe^+ and Al^+. Tropical soils that have been highly weathered contain Fe and Al hydroxides and oxides (sesquioxides); these act as bridges between the negatively charged clay particles and negatively charged organic particles. The formation of these microaggregates through anion-cation-anion complexes with SOM prevents leaching of cations (Jiménez and Lal, 2006), and contributes to the stabilization of OM by protecting organic compounds from rapid decomposition especially in hot climates (Nayak et al., 1990). Despite stabilizing SOC, these organo-mineral complexes at the same time reduce cation availability for plant uptake, particularly P which is limiting in these soils (Jiménez and Lal, 2006).

Not all tropical soils have the same texture, however. While Ferralsols have high clay and OM contents, Arenosols have high sand content (>900 g kg^{-1}) and are low in OM. This leads to low water retention, as well as high nutrient leaching, due to a higher proportion of large diameter pores (>30 µm) compared to more clay-textured soils (da Costa et al., 2013). In addition, Ferralsols and Acrisols contain mainly kaolinite clay, while Arenosols are dominated by quartz. These minerals have low CEC so that the presence of OM provides sites for cation exchange, improving nutrient levels and increasing the CEC (Glaser and Birk, 2012). Organic matter inputs to increase SOM levels in tropical soils can therefore help improve soil fertility by raising the CEC and lowering Al toxicity (Glaser et al., 2001). In sandy soils such as Arenosols in particular, OM inputs can lead to improved fertility, higher CEC, and higher WHC (Kasongo et al., 2011).

1.3.2 Climate

Along with physical and chemical properties of the soil, the input, decomposition and stability of SOM is particularly influenced by temperature and moisture (Swift et al., 1979). In strongly weathered, acidic tropical soils, such as those found in the Brazilian Amazon and Cerrado, litter decomposition rates are high, so litterfall contribution to SOM is low. Belowground input from root residue may thus be more important in these soils for SOM stability. The root residue's close contact with the mineral soil provides a greater chance of it being stabilized in soil aggregates compared to the litter (Oades, 1988; Zech et al., 1997). Differences in SOM levels within the same site can be explained by the chemical composition of the resources present, while external factors can help explain differences in a particular SOM pool across different sites (Zech et al., 1997).

Many ecosystem models have shown that decomposition rates increase with increasing temperature (Conant et al., 2011). In the field, however, the relationship between temperature and SOM is harder to observe, as other soil and hydrological conditions might have a more pronounced effect than temperature (Conant et al., 2011; Kalbitz et al., 2000). In addition, temperature may have different effects on different SOM pools which vary in size and decomposition rates (Conant et al., 2011). Liski et al. (1999), for example, observed that decomposition of older SOC is more resistant to temperature than litter. Incubation studies have mainly shown that slow decomposing OM is sensitive to temperature, but as these studies are necessarily short-term, they have focused on the 5-15% easily degradable portion of SOM (Conant et al., 2011). However, long-term field studies have also not been able to reach a consensus on the temperature-SOM decomposition relationship, since it is difficult to measure different belowground C inputs and the decomposition rates of different C pools, leading to various interpretations. Field studies also mostly focused on litter (Conant et al., 2011). Similarly, laboratory studies have shown that rising soil moisture increases soil heterotrophic respiration (and decomposition); however, the effect of moisture, like temperature, can also vary significantly (Ise and Moorcroft, 2006). This has implications for future scenarios that have been developed examining the effect of rising temperatures and altered rainfall patterns due to climate change on the C cycle. In addition, land-use conversion will also affect SOC accumulation depending on regional rainfall, soil type, and management practices (Batlle-Bayer et al., 2010).

1.3.3 Land management

Land-use conversion from forest to agricultural fields or pasture changes physical, chemical, and biological properties of the soil and can thus alter SOM stocks (Desjardins et al., 2004). In the case of converting forest to pastures, particularly with *Bracchiaria* grass species, studies in the Brazilian Amazonia have shown that SOC has either increased (d'Andréa et al., 2004; Koutika et al., 1997; Marchao et al., 2009; Morães et al., 1996), has stayed the same (Hetch, 1982; Serrão et al., 1979), or decreased (Desjardins et al., 1994). Proper pasture management has been observed to increase aboveground C by providing soil cover that reduces soil temperature and thus SOM decomposition (Batlle-Bayer et al., 2010). Soil texture can play a significant role on whether C stocks increase or not. Desjardins et al. (2004) observed that clayey soils in their central Amazonian study site contained higher C in the surface layer compared to sandy-clay soils in the eastern Amazonia site. Carbon levels decreased with depth (up to 20 cm), with more pronounced changes in the central Amazonia site than in the eastern site. Greater SOC loss has also been observed in coarse-textured soils in poorly managed systems (da Silva et al., 1994; Dieckow et al., 2009). Dieckow et al. (2009) noted that clayey soils were more resistant to SOM disturbances by conventional tillage than coarse-textured soils, while the latter showed to be more resilient.

Conversion of native vegetation to cropland under conventional tillage practices has been widely observed to cause high SOM losses (Guo and Gifford, 2002; Ogle et al., 2005; Puget and Lal, 2005). Conventional tillage buries surface crop residues in the soil (Tisdale et al., 1985), which helps incorporate organic matter in the soil, improve porosity, and control weeds (Hillel, 1982). However, extensive tillage causes high rates of soil erosion and breaks down the natural soil structure, which can lead to loss of SOM (Hillel, 1982; Montgomery, 2007). In addition, high-yielding soils that are not degraded require increasing amounts of inputs in order to maintain crop yields. Inefficient crop management through conventional practices thus leads to growing production costs as more expensive inputs are required to maintain high yields from nutrient-exhausted soils (Verhulst et al., 2010).

In contrast, an RMP that has been actively promoted in Brazil is no-till (direct seeding) which, if done properly, falls under the tenet of conservation agriculture (CA). CA's main objective is to protect the soil and cause the least amount of disturbance so as not to interfere with natural soil activities (Friedrich and Kienzle, 2008). Its three main principles are: 1) reduction in tillage, 2) retention of adequate levels (at least 30%) of crop residue on the soil surface, and 3) use of crop rotations (Ekboir, 2002; Govaerts et al., 2009). No-till can cause soil compaction and weed infestation which is why it is necessary that it be practiced along with surface residue retention and crop rotations (Govaerts et al., 2006; Teasdale et al., 2004). The combination of these best management practices leads to improved soil quality in terms of physical, chemical, and biological properties.

The importance of keeping crop residues on the soil surface highlights the contribution of OM inputs to SOM. The use of proper cropping systems and an appropriate fertilizer regime has been suggested as the best way to increase SOM levels in agricultural soils (Lal, 2009). Combining inorganic fertilizer applications with OM amendments, such as manure, can help increase SOC content in agricultural soils in the long-term (Purakayastha et al., 2008). In addition, OM amendments, particularly biochar, have been proposed as a method to retain and reduce loss of C (e.g. as CO_2 or DOC) from soils (Lehmann et al., 2006). As the quality of the OM entering the soil is also important (more labile sources decompose more quickly than more recalcitrant sources) (Angers et al., 2010), OM in the form of more resistant C, such as black C from biochar, could provide more stability to SOC, as well as DOC retention, in the soil.

1.4 Biochar

Black C is formed from burnt biomass caused either by natural fires or by man, such as charcoal and biochar. Biochar is differentiated from charcoal by the fact that it is made specifically as a soil amendment. Biochar is derived from waste biomass by pyrolysis, the thermochemical decomposition of OM at relatively low temperatures (<700°C) and in the absence of oxygen (Lehmann and Joseph, 2009). The use of pyrolysis to make both charcoal and biochar is called carbonization. This process has been present in civilization as long as there has been fire (Boateng et al., 2015). Traditional charcoal production, typically for the purposes of cooking and heating, centered on the use of charcoal pits and mound kilns, methods that persist until today due to their simplicity and low-cost (Brown et al., 2015). However, traditional charcoal production is highly polluting and potentially toxic, releasing carbon monoxide, methane, and volatile organic compounds (Brown et al., 2015; Shackley et al., 2015). Present-day production of charcoal is not the same as in the past when most of the world's population depended on it for energy. However, present technology is still highly inefficient, usually yielding charcoal from only 20% w/w of the original biomass (Boateng et al., 2015). Since biochar technology is still fairly recent, costs of production are not yet known definitively. Better carbonization and pyrolysis technologies need to be developed for the clean, efficient production of biochar (Shackley et al., 2015). Since biochar production is related to charcoal production, the following section discusses present charcoal production in Brazil and how biochar production can fit in.

1.4.1 Charcoal production in Brazil and potential for biochar production

Brazil is the largest charcoal producer in the world (approximately 9.9 Mt year^{-1}) (Boateng et al., 2015). The majority (80%) of the charcoal produced is for the metallurgical industry as an energy source for iron and steel production and as a thermo-reducing agent (Duboc et al., 2007;

FAOSTAT, 2011). The main problems caused by charcoal production are the high use of native vegetation as raw material and the greenhouse gases (GHG) emitted. From 2003 to 2012, 43% of raw material came from native vegetation and 57% from forest plantations, mainly eucalyptus and pine (CGEE, 2015). This places continued pressure on remaining native forests, particularly in the Cerrado (Duboc et al., 2007). In addition, the inefficient conversion of biomass into charcoal (presently less than 30% of the original biomass) leads to emissions of CO_2, carbon monoxide (CO), CH_4, and nitrogen oxides (NO, NO_2, and N_2O) (Duboc et al., 2007).

In 2010, the *Plano Siderurgia* (Metallurgy Plan) was launched with three objectives: 1) to reduce GHG emissions, 2) to avoid deforestation of native vegetation, and 3) to improve competitiveness of the Brazilian iron and steel industry based on a low C economy (CGEE, 2015). In a recent analysis of the plan, increasing production efficiency by as little as 5% was identified as the main path to reduce the use of native vegetation and GHG emissions. There are presently several companies in the Brazilian charcoal industry working to develop more efficient technologies, especially techniques that burn all gas emissions to produce energy that can be used for drying the raw material and for continued carbonization, as well as heating water. The condensed gases can also be collected as bio-oil and sold, adding more revenue to charcoal production, although as yet there is still little market for bio-oil (CGEE, 2015).

Charcoal in Brazil has been mostly produced in small circular, brick kilns, so-called “hot-tail kilns” (*rabo quente* in Portuguese). Hot-tail kilns release pyrolysis emissions (about 70% of the raw material) directly into the atmosphere, losing almost half of the wood biomass’ original energy (Bailis et al., 2013; de Miranda et al., 2013). In contrast, more efficient cylindrical, metal container kilns are being used by some producers because they burn wood faster and capture pyrolysis gases for use as co-products (Bailis et al., 2013). Yet, metal container kilns have been slower to be adopted due to the high initial investment compared to the brick kilns. As 70% of the charcoal produced in Brazil is by small-scale producers, public policies are required to improve access of these producers to new advances and equipment (CGEE, 2015). There are already several C reducing programs in place in Brazil through the *Banco Nacional de Desenvolvimento Econômico e Social* (BNDE; National Bank for Economic and Social Development). These include the *Programa Agricultura de Baixo Carbono* (ABC; Agricultural Low Carbon Program), BNDES *Meio Ambente* (BNDES Environment), BNDES *Florestas* (BNDES Forests), and a more charcoal-specific program, *Programa Fundo Clima – Carvão Vegetal* (Fundo Clima; Climate – Charcoal Fund Program). The Fundo Clima provides funding above R$10 million (~USD 3200 in 2017) for investments that improve charcoal kilns to above 35% charcoal yield, improve energy efficiency, and improve emissions recovery and treatment systems. Although these financial aids are available, many charcoal producers do not invest in updating their charcoal equipment due to current technological challenges for burning charcoal emissions and increased costs. It is therefore important for environmental agencies, both local such as the Fundo Clima or international such as the GEF (Global Environmental Fund), to provide incentives to reduce C emissions and provide other ways to profit, such as reusing the emissions for energy (CGEE, 2015).

As mentioned, several technological improvements in charcoal production are presently being actively explored in Brazil (Bailis et al., 2013; de Miranda et al., 2013). Within this context, Brazil shows great potential to become a leader in the development of biochar production as well. The same technological improvements made for charcoal production, mainly capturing gas emissions for energy production and collecting bio-oil, can also be applied for biochar production. Like the goals for improving charcoal production, biochar also aims, not only to improve soil conditions, but to mitigate C emissions and nutrient pollution, to assist in waste management, and to contribute to energy production (Lehmann and Joseph, 2015a). Producing biochar as a soil amendment could provide another incentive to charcoal producers as an extra source of income.

1.4.2 Biochar on soil chemical and physical properties

Biochar has shown potential to improve soil health and nutrient availability to plants, as well as enhance C sequestration from the atmosphere (Lehmann, 2007a). In addition, it may improve water infiltration and soil water retention (Ayodele et al., 2009; Laird et al., 2010). In contrast to other OM inputs to croplands and pasture such as manure, compost and mulches which are mineralized quickly and need to be applied annually, biochar can retain high amounts of C and fertility for many years (Lehmann and Rondon, 2002). Different types of biochar can be made depending on the feedstock and how it is made (low or high pyrolysis) (Spokas et al., 2012). The feedstock can determine the biochar's nutrient content and can be practically any sort of organic material, including weeds, crop residue, food waste, agro-industrial waste, wood waste, and others (Barrow, 2012). As many farmers typically burn crop residues, wastes and weeds on their fields, causing C emissions and contributing to DOC and dissolved black C (DBC), transforming these wastes into biochar to mix into the soil can be an effective way to sequester C while improving soil fertility (Barrow, 2012).

Questions still remain, however, about biochar regarding its performance, the processes that contribute to higher CEC after biochar addition, and the mechanism that biochar places on soil water retention (Sohi et al., 2001). In addition, reduced stability over a short period (decades) has been observed in both field and laboratory-made biochar. This could be attributed to production conditions (lower, e.g. 400°C-450°C, versus higher temperatures, e.g. 550°C-650°C) and type of feedstock which determine aromaticity in the biochar and thus its chemical resistance to decomposition (Fang et al., 2013). In addition to moving in dissolved form, black C may also be transported in particulate form, alone or with minerals, through macropores in the soil (Major et al., 2010). The important role of the physical protection of biochar-C in organo-mineral complexes also requires close examination in soils with different clay and OM contents (Fang et al., 2013).

1.4.3 Biochar and soil hydrology

Biochar applications can affect the soil hydrology as well, as it promotes mineral adsorption and can lead to increased soil aggregation, changes that may alter water flow in the soil (Major et al., 2012). Lehmann et al. (2003) observed that NH_4^+ leaching decreased significantly in central Amazonian Ferralsols amended with biochar. Potassium (K), however, was extremely mobile and rapidly leached, suggesting the need for slow-releasing K fertilizers or improving the soil's adsorption capacity. In a sandy clay loam Ferralsol of a Colombian savannah, Major et al. (2010) noted that a only a small amount of black C (0.45% of the applied biochar amount) had leached as DOC and as particulate organic C (POC) into the 150-300 cm layer below the 100 cm application layer, suggesting that black C was mainly adsorbed in the mineral layer. Increased water flux due to improved soil structure from biochar application (lower bulk density, improved saturated hydraulic conductivity, and water infiltration at the surface) were observed in both the 0-150 and 150-300 cm layers, which could explain the DOC and POC leaching. Bioturbation was also considered as a factor affecting black C movement below 150 cm, as earthworm burrows and termites were found in the experimental plots. Yet, since black C stocks were lower than applied rates after 2 years, but only a small amount was leached, it was suggested that most of the black C was lost through surface runoff. Major et al. (2010) conclude that biochar can contribute to the stable SOC sink and suggest high stability in soils once the labile black C fraction has been mineralized. High losses through surface erosion, however, suggest the importance of appropriate management practices. In this experiment, native savannah vegetation was cleared and the soil disked before adding biochar amendments. Aboveground vegetation was present, but it was not indicated how much litter there was. Combining surface residue and mulch with biochar applications could be useful in reducing runoff and preventing biochar and SOC losses.

The potential of biochar to increase water infiltration and retention in soils depends on its physical characteristics, which are directly influenced by the feedstock from which it is made. The different components of the organic material degrade at different temperatures: hemicellulose at 200 to 260°C, cellulose at 240 to 360°C, and lignin being the most resistant at 280 to 500°C (Sjöström, 1993). Wood biochars have been reported to have high organic C content (between 500 and 900 mg g^{-1} with increasing charring temperature), while manure and grass-based biochars can contain less than 500 mg g^{-1} organic C (Krull et al., 2009).

Although several production factors influence the physical properties of the final biochar (e.g. heating rate, pressure, reaction vessel, pre-treatment, post-treatment, and other parameters), the highest treatment temperature, or temperature of pyrolysis, is considered to be the most important determinant of a biochar's physical changes and thus its stability (Downie et al., 2009). Biochar aromaticity and recalcitrance is known to increase with increasing temperature (Downie et al., 2009; Lehmann et al., 2006). In addition, Mimmo et al. (2014) observed that WHC of biochars produced at lower temperatures (350-360°C) was lower than that of their original feedstock, with WHC increasing with increasing temperature of pyrolysis (>360). Similarly, Kinney et al. (2012) found that biochar field capacity (measured as mass water retained per mass of dry biochar), regardless of feedstock type (magnolia leaves, apple wood chips, and corn stover), was highest at temperatures of pyrolysis greater than or equal to 500°C. Microporosity of biochar has been demonstrated to increase with increasing temperature, contributing to greater water retention (Downie et al., 2009). Raising the temperature too high however, can lead to loss of surface area and porosity (Downie et al., 2009) as was observed by Brown et al. (2006) in pine-based biochar made at 1000°C compared to those at lower temperatures. Because of its high porosity, biochar can potentially improve soil porosity and soil water retention (Hardie et al., 2014), likewise retaining DOC. However, the role of biochar in retaining C in the form of DOC in tropical soils, specifically in Arenosols, has been little examined.

1.5 Dissolved organic carbon

DOC plays a key role in the flow of C through soil and may contribute to SOC pools in subsoils, yet is often overlooked in the global C budget. DOC consists of C from plants, animals, fungi and bacteria dissolved in a given amount of water, at a specific temperature and pressure. DOC compounds include soluble carbohydrates, amino acids, and complex high-molecular weight compounds. Easily identifiable compounds are fats, carbohydrates, and proteins, while more difficult to identify compounds are grouped as humic or fulvic (Jimenez and Lal, 2006).

Studies on DOC fluxes in the tropics are especially lacking. Although DOC in bodies of water receives more attention than DOC in soil, it is an important component of the C flux from terrestrial to aquatic ecosystems (Jimenez and Lal, 2006). It may also be a significant source of substrate for soil microorganisms (Jandl and Sollins, 1997) and is a “vector for the loss of C, N, and P from ecosystems” (p.31, Neff and Asner, 2001), thus affecting the ability for primary production in an ecosystem. Many field and laboratory studies have been carried out to examine

DOC dynamics; yet laboratory results often contradict those of field studies, mainly because the soil hydrology was not included (Kalbitz et al., 2000). In addition, the various methods for measuring DOC, including tension and zero tension lysimeters, sampling wells, and piezometers, can yield different results. However, it is difficult to separate differences due to methodology with underlying variability caused by soil characteristics and vegetation (Neff and Asner, 2001). Understanding how DOC and its nutrients are transported and removed from the soil in the field, where they can potentially affect microorganisms in downstream waters, therefore requires further examination (Qualls and Haines, 1992).

Vertical movement of DOC through the soil profile often leads to more labile DOC in the litter layer and less biologically available DOC in deeper soil as it moves down. This may be due to either the transport of more recalcitrant DOC or to the physical desorption of C from SOM to DOM (Qualls and Haines, 1992). In a laboratory study with samples from an oak-hickory forest, Qualls and Haines (1992) observed that biodegradability of DOM from throughfall (water falling from canopy leaves) to the A horizon decreased, but then increased in the mineral horizons. In general, decomposition of the soil-solution and stream-water DOM was very slow, with the A horizon being particularly stable. Qualls and Haines (1992) conclude that DOM was rapidly adsorbed in the A horizon, preventing it from being removed from the soil matrix and percolating further down. Biological mineralization of the adsorbed DOM would occur very slowly over a long period of time. Many field studies have corroborated that DOC adsorption is likely the main process of its stabilization in the mineral layers (Kalbitz et al., 2000).

Similarly, Johnson et al. (2006) observed in south-western Brazilian Amazonian soils that DOC concentrations decreased with depth, with higher concentrations in quickflow compared to deeper hydrologic flows. This implies that DOC in these tropical soils is mainly transported to aquatic ecosystems by surface flow rather than percolating into deeper layers (Johnson et al., 2006). Likewise, Marques et al. (2012) reported that the clayey Ferralsols of central Amazonia retained higher DOC concentrations at the surface, with decreased concentrations and flow in deeper soils. According to Pinheiro et al. (2004), a decrease in organic C levels in tropical soils may be related to reduced aggregate stability, leading to nutrient losses and erosion.

Land-use changes such as clear-cutting, conversion of native vegetation to cropland, adding fertilizers and liming, can affect DOM dynamics by changing amounts of OM input and substrate quality, as well as altering microbial decomposition and stabilization of OM (Cronan et al., 1992). A soil's ability to protect SOM from microorganisms and retain cations may be "an important factor in the amounts and fluxes of [DOC] in soils" (p.350, Jiménez and Lal, 2006), since DOC is mainly released through microbial activities (Guggenberger et al., 1994). In a study examining the conversion of native forest to plantations in a subtropical forest region of China with loam-textured Ferralsols, Wu et al. (2010) observed that the removal of the topsoil, lack of fertilization in the last 5 years before sampling, and the subsequent reduction in biomass production and litterfall in a chestnut forest plantation led to a decrease in water-soluble organic C, i.e. DOC. In the bamboo forest plantation, applications of straw and rice husk mulch during the winter contributed to higher DOC concentrations in certain months compared to the native forest and chestnut forest, but higher in the native forest than in the plantations in other months, reflecting a strong seasonal, as well as management practice, effect.

DOC dynamics thus can vary considerably depending on rainfall amount, seasonality, soil texture, vegetation cover, climate, and land-use. Yet one can conclude that highly weathered tropical soils with low SOM are vulnerable to changes in land-use that remove the native vegetation and disturb the soil, breaking apart protective soil aggregates. In addition, the presence of metals (e.g. Fe, Al) that bond readily with clay particles limits the availability of important nutrients, such as P, necessary for plant growth, requiring fertilizer application and proper crop management to ensure continued productivity. Since most of the SOM and DOM are retained in the surface layers, DOC is most easily transported through surface flow and thus removed through runoff and erosion. RMPs such as minimum to no-till, retention of residue cover, and OM inputs, such as biochar, can assist in reducing loss of C through DOC in runoff and protecting the physical stability of SOM. However, biochar amendments to tropical savannah soils have either shown decreased (Eykelbosh et al., 2015) or increased (Major et al., 2010) DOC leaching, suggesting that DOC retention will vary depending on biochar and soil type.

1.6 Research questions

To better understand the influence of biochar additions to soil fertility, to DOC, and to soil hydrology in Cerrado Arenosols, greenhouse experiments were conducted for this thesis comparing biochars made from different feedstocks: cotton residue, swine manure, eucalyptus residue, and sugarcane filtercake, pyrolized at different temperatures. The following questions were addressed:

1) What are the effects of biochar feedstock type and temperature of pyrolysis on maize biomass and soil water retention in a Cerrado Arenosol soil? **(Chapter 2)**

> **Hypothesis:** Maize biomass and soil water retention will be greatest in higher temperature biochar treatments for all feedstocks. Higher temperature biochars have been associated with greater porosity.

2) How does biochar feedstock and application rate impact soil nutrient concentrations and maize physiology? **(Chapter 3)**

> **Hypothesis:** Higher biochar application rates will contribute to higher soil nutrient levels and improved maize growth. Swine manure biochar will lead to the greatest maize biomass because raw animal manures are usually high in nitrogen and other available nutrients.

3) What are the effects of biochar feedstock type and temperature of pyrolysis on DOC quality and leaching? **(Chapter 4)**

> **Hypothesis:** Eucalyptus biochars will retain DOC the most and will have the largest increase in soil C levels due to its feedstock's high C/N ratio, allowing the formation of more organo-mineral complexes to protect DOC. Higher temperature biochars are more stable and therefore will reduce DOC losses, particularly of humic DOC.

1.7 Significance

Considering tropical soils hold high amounts of SOC (~30% of the global SOC pool) (Jiménez and Lal, 2006) and are undergoing extensive land-use conversion, developing management practices that can stabilize SOC stocks and improve soil resilience to changing regional climate is essential. In addition, as tropical soils such as Cerrado Arenosols, are transformed into cropland and pasture, their conservation to maintain productivity is key to the regional economy. In the state of Mato Grosso, which encompasses the Brazilian Cerrado (Figure 1.1), Arenosols account for 13% of the state's area (about 11.7 million ha) (SEPLAN, 2008) (Figure 1.2). A large portion of this soil is under maize production during the dry season, a crop that has become increasingly important to the state's economy (IBGE, 2016). As charcoal production is already significant in Brazil, integrating biochar production to transform locally available waste biomass into more stable organic matter amendments could provide additional incentive for GHG-reducing programs, particularly if the biochars improve crop yields.

Figure 1.2. Arenosols (dark grey) in the state of Mato Grosso, Brazil (adapted from SEPLAN, 2001). Cuiabá is the state capital.

For these reasons, this thesis proposes three research questions aimed at better understanding the contribution of biochar as a soil amendment to improve the physical and chemical properties of a Cerrado Arenosol. Moreover, the biochars used were produced of locally available waste residue to test the quality of the feedstocks as soil amendments; if beneficial their conversion to biochar may reduce both waste volume and C emissions to the atmosphere. The unique contributions of this thesis include the use of fluorescence spectroscopy with parallel factor analysis of excitation-emission matrices to examine DOC quality in biochar-soil leachate, and an examination of water retention in a Cerrado Arenosol mixed with different biochars (varying feedstocks and temperatures of pyrolysis) under maize. In addition, biochar contribution to maize production is of interest to producers in the state of Mato Grosso seeking to maintain soil productivity and crop yield. Use of biochar could potentially lead to reduction in lime and fertilizer costs, if the cost of biochar is less than that of lime (Jeffery et al., 2017). Installation of an efficient on-site biochar reactor could also reduce waste transportation and on-farm energy costs, while indirectly contributing to climate change mitigation through C sequestration.

Chapter 2: Biochar effects on maize growth and soil water retention in a Brazilian Cerrado Arenosol

2.1 Introduction

The Cerrado is the second largest biome in Brazil, covering 24% of the country's area (Bustamante et al., 2012). The region's natural vegetation consists of a variety of vegetation types which vary structurally and in species composition (Arantes et al., 2016). The Cerrado is considered a global hotspot of biodiversity (Mittermeier et al., 2005), in particular for plant diversity (Mendonça et al., 2008). Yet, despite its ecological importance, the region has been experiencing rapid deforestation and conversion of natural grassland ecosystems since the 1970s with replacement by exotic grasses (mainly *Brachiaria* sp.) for cattle-raising, and conversion to croplands for soybean, maize, and bean production (Arantes et al., 2016). These land-use changes have significant environmental implications since they lead to lower carbon stocks, higher greenhouse gas emissions, lower evapotranspiration, and increased heat flux. The reduced evapotranspiration can ultimately lead to decreased regional rainfall (Arantes et al., 2016).

This regional climate change would greatly affect the regional economy, including that of the state of Mato Grosso which encompasses a significant portion of the Cerrado. In Mato Grosso, the maize crop planted after the soybean harvest accounts for 98% of all the maize grown in the state (IBGE, 2016). This crop is typically cultivated towards the end of the rainy season (February), using residual soil moisture, and harvested in the dry season (usually in June). For this reason, dry season maize is particularly vulnerable to changes in precipitation patterns (Cruz et al., 2010). In addition, most of the maize is produced on sandy soils, Arenosols. Accounting for 13% of the area of the state of Mato Grosso (about 11.7 million ha) (SEPLAN, 2008), Arenosols are low in organic matter and their high sand content causes poor water retention (da Costa et al., 2013), leaving crops vulnerable to droughts.

Biochar (charcoal derived from waste biomass by pyrolysis) has been observed to increase soil water and nutrient retention under some conditions (Abel et al., 2013; Lehmann, 2007b; Sohi et al., 2009; Spokas et al., 2012), and so amending sandy soils with biochar could potentially be beneficial in this system. As biochar is very porous, it can lead to improved soil porosity, soil water content, plant available water content (AWC), soil bulk density, and soil hydraulic conductivity (K) (Barnes et al., 2014; Hardie et al., 2014; Uzoma et al., 2011b). In addition, it can affect electrical conductivity (EC), with the biochar's EC tending to increase with increasing temperature of pyrolysis (Gray et al., 2014). However, as with other agronomic contributions, biochar may also have either no effect or a negative effect on soil water and nutrient retention depending on the biochar and soil (Kinney et al., 2012; Masiello et al., 2015; Streubel et al., 2011). For this reason, it is necessary to more closely examine the soil physical properties related to soil hydrology that can be affected by biochar addition, including soil bulk density, porosity, and grain size distribution (Masiello et al., 2015). To better understand biochar effects of water retention on crop production in a sandy Cerrado soil, a greenhouse experiment was conducted to assess maize growth in an Arenosol mixed with biochars produced from different agricultural waste feedstocks and temperatures of pyrolysis. In addition, biochar properties, alone and mixed with soil, were examined to observe how they may alter soil water retention, hydraulic conductivity, and EC, in turn affecting maize biomass. The hypothesis was that higher temperature biochars have greater porosity and thus they would improve soil water retention, leading to greater maize biomass.

2.2 Materials and methods

2.2.1 Soil collection and biochar production

Soils from the top 0-20 cm layer were collected from an agricultural field located within the farm Fazenda Água Azul (15°13'55.2"S, 54°57'43,4"W) managed by the agribusiness Grupo Bom Futuro, 178 km northwest of the state capital of Cuiabá in Mato Grosso, Brazil, an area within the Cerrado biome. The soil collected was classified as an Arenosol (FAO soil classification), with a sandy texture (91% sand, 4% silt, 5% clay). Carbon (C) and nitrogen (N) levels in the soil were 0.7 % C and 0.08 % N as determined by elemental analysis (628 Series CHN Analyzer, LECO Corp., St. Joseph, MI). The average pH_{water} was 5.8 and average CEC was 5.3 $cmol_c\ kg^{-1}$, with a bulk density of 1.6 $g\ cm^{-3}$. Over the last 10 years, the crops sown on the study site included soybean, sorghum, maize, and cotton, with the latter two crops grown in rotation with soy for the last three years (Afonso Campos da Silva, Grupo Bom Futuro, personal communication). Twelve biochars were commercially produced (SPPT Ltda., Mogi Morim, São Paulo, Brazil) from four feedstock materials: cotton husks, eucalyptus sawmill residue, sugarcane filtercake, and swine manure, slow-pyrolyzed at three temperatures (400°, 500°, 600°C). These were subsequently crushed and sieved to <2 mm in order to have similar biochar particle sizes between the different feedstocks and similar to soil particle size.

2.2.2 Experimental design

In a greenhouse located at the Federal University of Mato Grosso (UFMT), Cuiabá campus, 9 L pots (24.5 cm top diameter, 20 cm bottom diameter, 22.7 cm height) with one hole (3 mm) drilled in the bottom received 8 kg of an air-dried, sieved (<2 mm) Arenosol. The greenhouse temperature was controlled to 28±2°C, similar to temperatures during which the dry season maize is grown from January to June (INPE, 2012). Biochar was applied to soil in pots at 5% soil dry weight, and mixed and compacted by hand in each pot. The pots were divided into 4 blocks, with each block running north-south along a greenhouse bench, with a replicate of each treatment (biochar amended soil) plus a control (unamended soil) randomly assigned to locations within each block. Water was initially added to achieve field capacity and allowed to equilibrate.

Since fertilizer applications are a standard management practice in the region, fertilizer was added to the pots corresponding to the amount each maize plant requires at the rate of 150 kg NPK+S, 150 kg KCl and 200 kg urea for 60,000 plants/hectare in the field (Afonso Campos da Silva, Grupo Bom Futuro, personal communication, 2014). Thus, after one week, 2.5 g of crushed NPK+S (12-46-0 + 7) was added to the center of each pot. Four maize seeds (DKB 390 VT PRO2 variety, Dekalb) were planted in all pots around the center where the fertilizer was added so that roots would have room to spread all around. After 20 days, 2.5 g of crushed KCl and 2.0 g of urea diluted in 50 mL of water were added, followed by another 1.3 g of diluted urea applied 7 days later. Application times mimicked those of nutrient management strategies utilized by farm managers, but modified to suit the shorter growing period used in this greenhouse experiment. Pots were watered three times a week to maintain soil moisture at 60% of field capacity for 45 days. Volumetric water content (θ) and EC were directly measured and recorded once a week using a GS3 sensor (Decagon Devices, Inc., Pullman, WA, USA). At the end of the experiment, above and belowground maize biomass was collected, weighed fresh, then dried at 60°C for 48h and reweighed. Soil samples were analyzed for macronutrient (available P, K^+, Ca^{2+}, Mg^{2+}, and S) and micronutrient (Zn, Cu, Fe, Mn, B) availability according to the standard soil methodologies used by the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, 2009) as described in Eykelbosh et al. (2014). Soil total C and N were analyzed on a CHN Analyzer (628 Series, LECO Corp., St. Joseph, MI).

2.2.3 Water retention curves

At the end of the experiment, intact soil cores (100 cm^3) were taken from each pot, resulting in 52 cores to be used in the laboratory. A fine mesh was placed at the bottom of each soil core and the cores placed in a pan of water to saturate for 24 h before placing them in a tension table to determine θ at 0, 2, 4, 6, 8, and 10 kPa (Reinert and Reichert, 2006). Afterwards they were transferred to pressure chambers to determine θ at 33 and 100 kPa. The samples were kept at each matric potential for one week then weighed before moving to the next matric potential. θ at 500, 1000, and 1500 kPa was determined using the WP4C Dewpoint Potentiameter (Decagon Devices, Decagon Devices, Inc., Pullman, WA, USA) (Klein et al., 2006), as described by Eykelbosh et al. (2014). θ ($cm^3\ cm^{-3}$) for all matric potential points (0 – 1500 kPa) was then entered into the Soil Water Retention Curve software (SWRC, version 2.0, University of São Paulo, São Paulo, Brazil) (Dourado-Neto et al., 2000) to adjust the soil water retention, θ, of each replicate using the unimodal constrained model of van Genuchten (1980):

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{[1+(\propto\psi_m)^n]^m}$$

where $m = 1 - 1/n$ (Mualem, 1976), θ is volumetric water content, ψ_m is matric potential, θ_r is residual θ, θ_s is saturated θ, and n and α are adjusted parameters. The results were then used to obtain AWC and water retention curves for each treatment. AWC (%) was calculated as θ at 33 kPa (field capacity) minus θ at 1500 kPa (permanent wilting point). Final bulk density was also determined from intact soil cores.

2.2.4 Hydraulic conductivity measurements

In addition to the measurements provided by the tension table, pressure chambers, and WP4, saturated and unsaturated hydraulic conductivity (e.g. K(θ)) was determined in the laboratory using the HYPROP® (UMS GmbH, Munich, Germany), which employs the simplified evaporation method (Peters and Durner, 2008; Schindler et al., 2010). The HYPROP® holds two vertically aligned tensiometers (bottom and top) with ceramic cups at the end and takes measurements as the soil water evaporates. An Arenosol (sifted to <2 mm) was mixed dry with each biochar at 5% (w/w), moistened to 20% θ and packed into 250 cm^3 stainless steel cores (8 cm diameter, 5 cm height) to a bulk density of 1.2 g cm^{-3}. A fine mesh was applied at one end and the core placed in a pan of water to saturate for 24 h. The HYPROP® sensor head and tensiometers were refilled with distilled water manually using the syringe method and allowed to sit for 24 hours to degas as much as possible. After 24 hours, two holes for each tensiometer were drilled in the saturated soil sample, the sample placed onto the HYPROP® sensor head with the tensiometers in place, and the HYPROP® with sample was set on a scale. Both the HYPROP® device and the scale were connected to a computer running the tensioView® software (version 1.10, UMS GmbH, Munich, Germany). Weight and tensions (Ψ) were recorded automatically by tensioView® as the soil dried by evaporation in the laboratory environment. Once air entered the ceramic cup of the tensiometers (after 7 to 10 days) and the Ψ readings dropped to 0 kPa, the measurement was concluded. The soil was then removed from the core into a dish and placed in the oven to dry at 105°C for 24 hours and weighed.

The soil dry weight was entered in the HYPROP-FIT software to calculate the θ during the measurements. Using HYPROP-FIT, the retention curve, $\theta(\Psi)$, and hydraulic conductivity, $K(\theta)$, were determined by fitting the data to the van Genuchten (1980) model for the retention curve and the Mualem (1976) model for the conductivity curve. The software also provided the quality of the fit to the model by root mean squared error (RMSE) for both θ and the log of hydraulic conductivity, K, along with parameter values. This procedure was repeated for the 400 and

600°C biochar-soil mixtures. As measurements for each biochar lasted about a week and could only be measured one sample at a time, it was not feasible to include all 12 biochars with replicates. The HYPROP® results thus serve to provide the potential water retention characteristics and an estimate of the hydraulic conductivity of soil mixed with the biochar feedstocks at a high and low temperature of pyrolysis and compare the results to that of the tension table, pressure chambers, and WP4.

2.2.5 Biochar particle size and porosity analysis

For particle size analysis in the laboratory, soil samples collected at the end of the experiment were separated into sand (>53µm) and silt+clay (<53µm) fractions following the fractionation method by EMBRAPA (1997). Briefly, sodium hydroxide (NaOH) and distilled water were added to 10g of air-dried soil and shaken overnight. After this time, sand and silt+clay fractions were separated using a 53µm sieve. Once separated, an aliquot of the silt-clay fraction in suspension was placed in a laser diffraction particle size analyzer (LA 950, Horiba Scientific, Edison, NJ) to determine particle size. Afterwards, particle size of the oven-dried sand fraction was measured. Particle size of each biochar alone (< 2 mm) was determined directly by the analyzer.

The Brunauer-Emmett-Teller (BET) total surface area (Brunauer et al., 1938), total pore volume, Barrett-Joyner-Halenda (BJH) mesopore surface area and volume (Barrett et al., 1951), deBoer t-plot micropore surface area, and micropore volume of each biochar were determined from automated gas sorptometry with N_2 performed by an ASAP 2020 Plus Physisorption Analyzer (Micromeritics, Norcross, GA, USA). Results of the physical characterization of the biochars are shown in Table 4.3.

2.2.6 Statistical analyses

The effects of biochar treatments on plant biomass, soil nutrients, θ, AWC, EC, bulk density, and particle size were determined by univariate analysis of variance (ANOVA) and multivariate (MANOVA) for grain size distribution (D10 and D90), using IBM® SPSS® Statistics software (version 23, SPSS. Inc., Chicago, USA). Where treatments were significant, a post-hoc Tukey test ($P < 0.05$) was used to compare means, and a post-hoc Games-Howell ($P < 0.05$) test when variances were unequal as in the case of particle size and distribution. Pearson correlations and linear regressions were performed between plant biomass and mean θ, EC, and AWC, as well as between mean θ and EC. Principal component analysis (PCA) was carried out on soil chemical and physical properties in biochar treatments using the FactoMineR package (Le et al., 2008) in R (version 3.3.1). Values presented in graphs are means ±1 standard error (SE). RMSE values for θ and K determined by HYPROP-FIT indicate the extent of agreement between the predicted and measured values; the smaller the RMSE, the better the fit between the values (Shwetha and Varija, 2015).

2.3 Results

2.3.1 Maize biomass

After 6 weeks, filtercake biochar had the highest mean above and belowground dry biomass at 600 °C, with dry aboveground biomass (16.7±0.9) significantly ($P < 0.05$) higher than the control (11.8±0.4) (Figure 2.1A). For both filtercake and eucalyptus biochars, mean aboveground biomass increased with increasing temperature, while for cotton and swine manure biochars mean plant biomass decreased with increasing temperature. Maize biomass was significantly ($P < 0.05$) lower in soils with cotton and swine manure biochars compared to eucalyptus and sugarcane filtercake biochars and the control (soil without biochar) (Figure 2.1). Aboveground dry biomass in soils with swine manure biochar at 400 °C was significantly higher than at 600 °C (Figure 2.1A).

Aboveground dry biomass in eucalyptus biochar treatments did not differ between the temperatures (Figure 2.1A), and belowground dry biomass also showed no significant differences between the temperatures for any of the feedstocks (Figure 2.1B). Analysis of soil macronutrients showed that soils with cotton and swine manure biochars had the highest potassium (K) and sulfur (S) levels compared to the other biochars, but the lowest calcium (Ca) levels (Figure 2.2). Both K and Ca were strongly ($P < 0.001$) correlated with aboveground dry biomass: K negatively correlated (R = −86), while Ca positively correlated (R = 0.74) (Appendix Figure A.1). The Ca/Mg ratio was also highest in eucalyptus and filtercake biochar treatments compared to cotton and swine manure biochars (Appendix Figure A.2). For soil micronutrients (Zn, Fe, Cu, Mn, B), there were significant differences between the biochar feedstocks, but no differences between temperatures for each feedstock (Appendix Figure A.3).

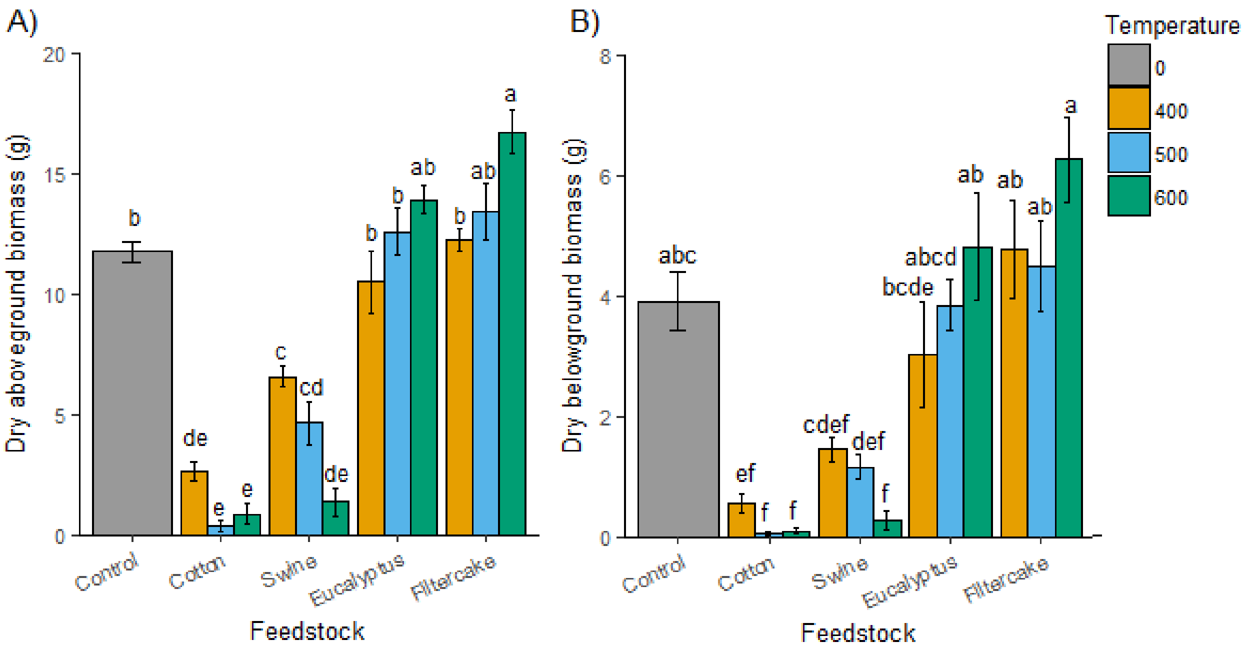

Figure 2.1. A) Mean dry aboveground biomass (g), and B) mean dry belowground biomass (g). Different letters represent significant differences between the biochar treatments including the control (n=4, Tukey test; $P < 0.05$).

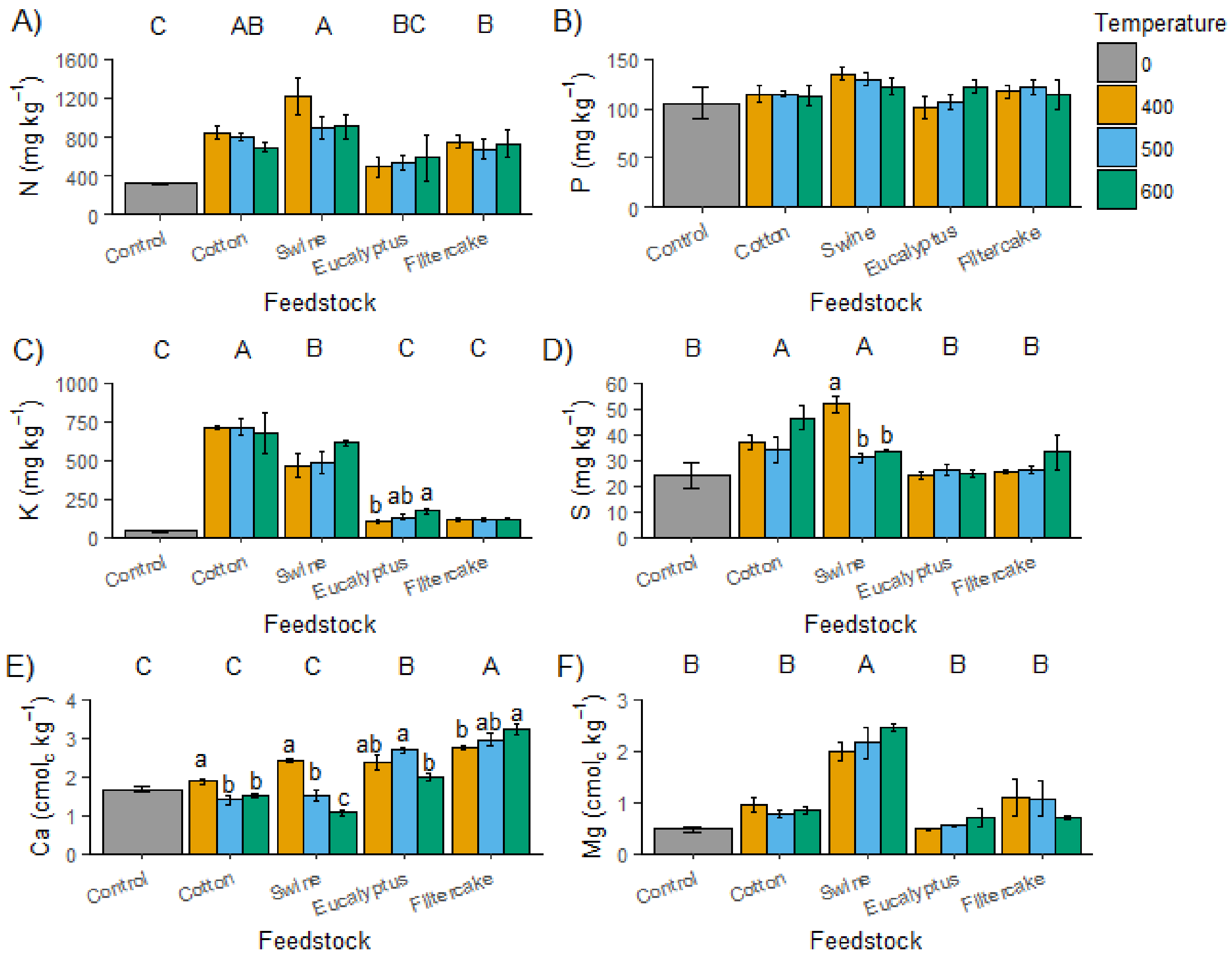

Figure 2.2. Mean concentration of A) soil total nitrogen (N; mg kg^{-1}) and available soil macronutrients: B) phosphorus (P; mg kg^{-1}), C) potassium (K; mg kg^{-1}), (D) sulfur (S; mg kg^{-1}), (E) calcium (Ca; cmol$_c$ kg^{-1}), and F) magnesium (Mg; cmol$_c$ kg^{-1}) in soils with different biochars (n = 4). Capital letters indicate significant differences between the feedstocks and lowercase letters indicate significant differences between the temperatures for each feedstock (Tukey test, $P < 0.05$); where absent, differences were not significant.

2.3.2 θ and EC measurements

Mean weekly θ measurements showed that cotton and swine manure biochar treatments had significantly ($P < 0.05$) greater θ than eucalyptus, filtercake, and control treatments. Only swine manure biochar had significant differences between the temperatures, where θ of soils with swine manure biochar at 600°C was greater than that at 500°C (Figure 2.3A). EC measurements were similar, cotton biochar treatments having the highest mean EC, followed by swine manure biochar, and lastly eucalyptus, filtercake, and control treatments which were not different from each other. The differences between temperatures for swine manure and eucalyptus biochars were similar as observed for θ. Cotton and filtercake biochars, however, had significant differences, with EC in soils with cotton biochar at 600°C significantly greater than that at 400°C while the opposite was observed for soils with filtercake biochars (Figure 2.3B).

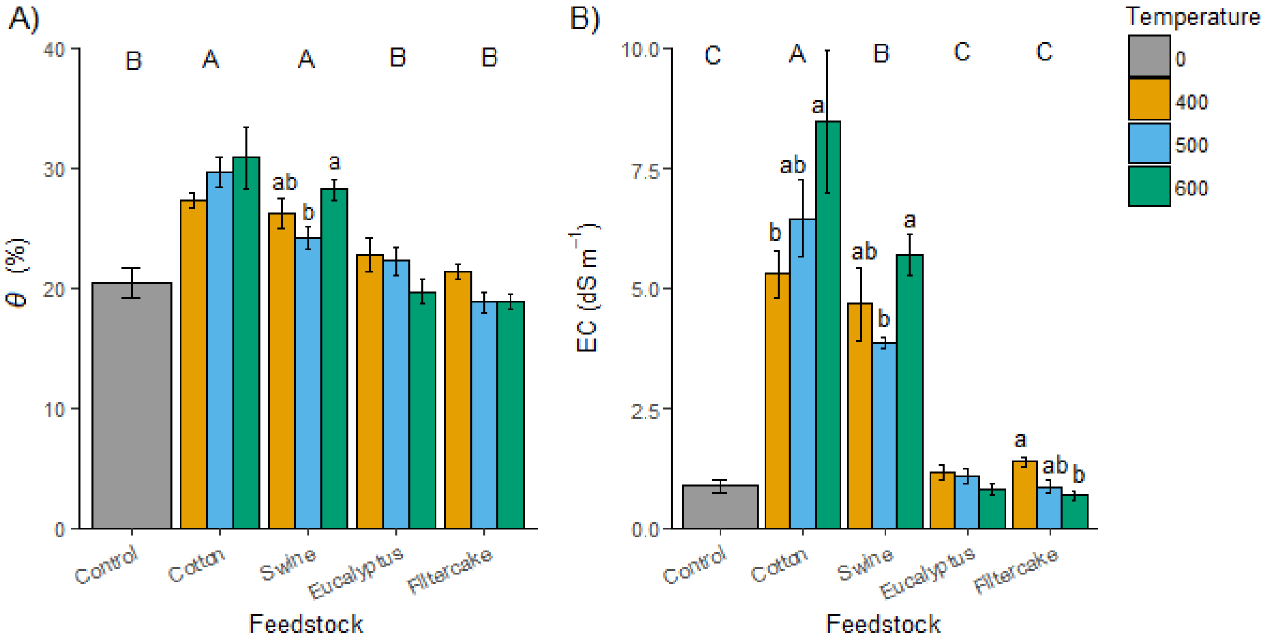

Figure 2.3. A) Mean volumetric water content (θ, %) and B) electrical conductivity (EC, dS m^{-1}) over 6 weeks (*n* = 4). Capital letters represent significant differences between the feedstocks while lowercase letters represent significant differences between the temperatures (Tukey test; $P < 0.05$).

The correlation between EC and θ showed a positive relationship between the two parameters, with an R of 0.92 ($P < 0.001$). Plant biomass was also significantly and negatively correlated with both mean θ and mean EC, as well as AWC (Table 2.1), with biomass decreasing with increasing θ, EC, and AWC.

Table 2.1. Pearson correlations between final maize biomass and mean θ (%), EC (dS m^{-1}), and AWC (%) over 6 weeks. * =*P* <0.001**

	θ (%)	EC (dS m^{-1})	AWC (%)
	R	**R**	**R**
Aboveground dry biomass	-0.88***	-0.88***	-0.71***
Belowground dry biomass	-0.83***	-0.80***	-0.62***

2.3.3 Water retention

Water retention curves determined from the intact soil cores reflected mean *θ* for each treatment (Figure 2.4). Cotton biochar treatments had higher water retention, followed by swine manure, eucalyptus, and filtercake biochars, which were almost indistinguishable from each other. The control treatment had the lowest water retention.

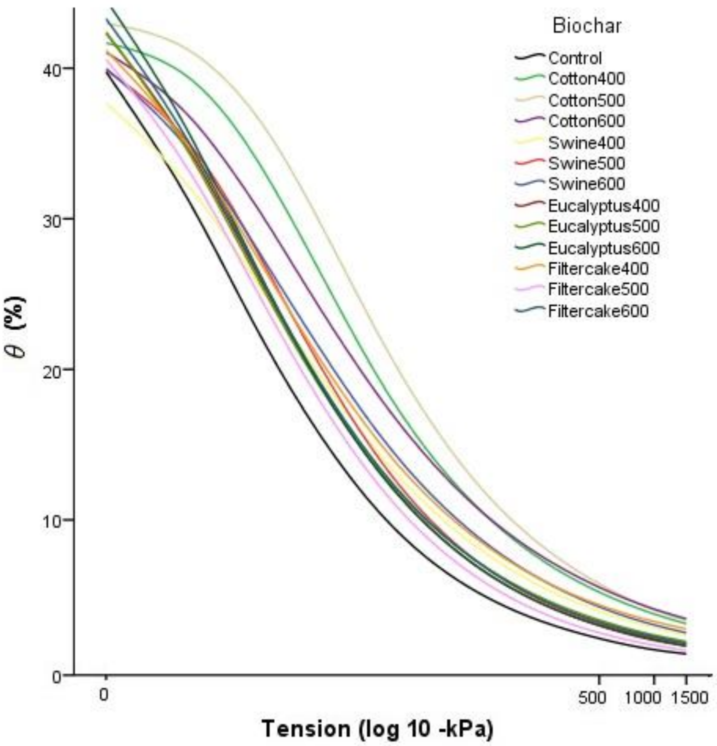

Figure 2.4. Water retention curves of intact soil cores (*n* = 4).

For AWC, cotton and swine manure biochar treatments had the highest levels and were significantly ($P < 0.05$) different from the control, but swine manure biochar did not differ from eucalyptus and filtercake biochars. The latter were also not significantly different from each other or the control. Only cotton biochar had significant differences between the temperatures, with AWC higher in soils with 500°C cotton biochar than in soils with 600°C biochar (Figure 2.5).

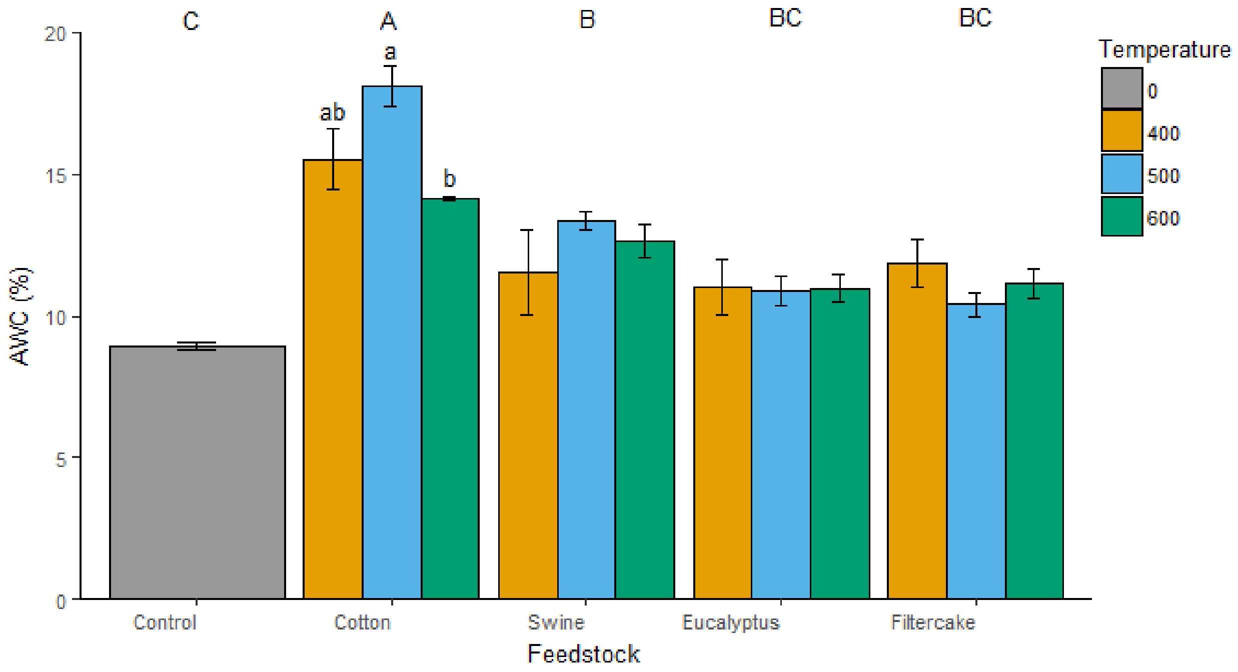

Figure 2.5. Mean available water content (AWC, %) determined from intact soil cores (*n* = 4). Capital letters represent significant differences between the feedstocks while lowercase letters represent significant differences between the temperatures (Tukey test; *P* < 0.05).

Final bulk density determined from intact soil cores showed no differences between the temperatures for each feedstock, and little difference between the feedstocks. Although most of the biochar treatments had lower mean bulk density than the control, only the bulk densities of soil with cotton biochar at 600°C and eucalyptus biochar at 600°C were significantly lower (Table 2.2).

Table 2.2. Bulk density from intact soil cores. Lowercase letters indicate significant differences between the treatments (*n* = 4, Tukey test, *P* < 0.05) for each experiment.

Treatment	**Bulk density (g cm^{-3})**
Control	1.4±0.03 a
Cotton400	1.2±0.05 abc
Cotton500	1.2±0.03 abc

Treatment	Bulk density (g cm^{-3})
Cotton600	1.2±0.04 bc
Swine400	1.3±0.04 abc
Swine500	1.4±0.03 ab
Swine600	1.3±0.00 abc
Eucalyptus400	1.3±0.05 abc
Eucalyptus500	1.2±0.03 abc
Eucalyptus600	1.2±0.03 c
Filtercake400	1.3±0.04 abc
Filtercake500	1.3±0.03 abc
Filtercake600	1.3±0.03 abc

2.3.4 Particle size analysis

2.3.4.1 Biochar characteristics

Particle size did not vary much between the biochars, except for the particle size of filtercake biochars, which was significantly lower than the others (Table 2.3). Between the temperatures of pyrolysis for each feedstock, there were no differences for cotton and swine manure, while filtercake at 400°C had larger particle size than at 500°C, and eucalyptus 400°C and 600°C larger than at 500°C [1] (Table 2.3). On examining the finest (D10) and coarsest (D90) parts of the grain size distribution (Horiba Scientific, 2012) between feedstocks, filtercake biochar had the lowest D90, while there were no differences in D10. There were differences between the temperatures of pyrolysis for each feedstock for both D10 and D90, but no consistent trend.

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[1] In other analysis of physical characteristics (not shown), eucalyptus biochar at 500°C has appeared similar to filtercake biochars, suggesting it may have been cross-contaminated with filtercake during production.

Table 2.3. Porosity determined by BET-N_2 sorption (n = 1) and particle size and distribution of 12 biochars (4 feedstocks x 3 temperatures of pyrolysis). Micropores are identified as pores <2nm diameter, mesopores as pores between 2 and 50nm diameter. For particle analysis, capital letters indicate significant differences between the feedstocks and lowercase letters between the temperatures of pyrolysis for each feedstock (n = 3; Tukey test, P < 0.05

Biochar	Total surface area (m^2 g^{-1})	Mesopore area (m^2 g^{-1})	Micropore area (m^2 g^{-1})	Total pore volume (cm^3 g^{-1})	Mesopore volume (cm^3 g^{-1})	Micropore volume (cm^3 g^{-1})		Particle size (µm)		D10 (µm)		D90 (µm)
Cotton400*†	0.2	n/a	n/a	0.0017	n/a	n/a	A	888.2±131.1 a	A	33.0±7.3 ab	A	2790.5±20.3 a
Cotton500	1.8	0.47	2.4	0.0056	0.0050	0.0012		757.2±27.3 a		24.6±1.3 b		2747.6±24.7 a
Cotton600	1.9	0.53	2.9	0.0061	0.0055	0.0014		806.4±53.8 a		53.7±7.2 a		2715.9±19.3 a
Swine manure400	7.2	4.1	0.7	0.0323	0.0289	0.0002	A	966.3±94.6 a	A	210.0±8.9 a	A	2733.8±0.03 a
Swine manure500	24.9	9.6	9.7	0.0725	0.0596	0.0046		779.2±51.9 a		61.0±2.2 a		2706.6±10.7 a
Swine manure600	36.9	10.1	15.4	0.0715	0.0524	0.0073		822.7±25.2 a		143.2±40.6 a		1606.7±3.3 b
Eucalyptus400†	0.3	n/a	2.3	0.0003	n/a	0.0011	A	860.6±43.9 a	A	47.4±7.9 a	A	2808.7±10.0 a
Eucalyptus500	42.3	4.9	31.2	0.0520	0.0312	0.0151		415.6±43.5 b		37.3±3.4 a		476.9±38.6 b
Eucalyptus600*†	132.0††	n/a	n/a	0.07	n/a	n/a		965.5±71.8 a		45.5±6.9 a		2824.3±6.1 a
Filtercake400	13.5	10.3	0.4	0.0851	0.0787	-0.0002	B	457.9±7.3 a	A	42.4±1.6 a	B	1241.6±61.7 a
Filtercake500	25.0	14.6	5.1	0.1210	0.1095	0.0021		215.4±37.9 b		31.1±0.7 b		601.7±156.8 a
Filtercake600	41.3	17.6	12.7	0.1314	0.1112	0.0059		363.2±65.2 ab		38.4±2.9 ab		947.9±212.4 a

Correlation between biochar particle size and D90 showed a strong positive correlation (R = 0.88; $P < 0.001$). Particle size and total pore volume also showed a significant yet negative correlation (R= -0.69; $P < 0.001$), with total pore volume decreasing with increasing particle size.

2.3.4.2 Biochar-soil mixtures

The sand aggregate size of soils with filtercake biochar was significantly greater ($P < 0.05$) than that of cotton and swine manure biochars, but not different from soil with eucalyptus biochar (Table 2.4). Sand aggregate size of soils with eucalyptus, cotton, and swine manure biochars did not differ between each other, and soils with all biochars had significantly greater ($P < 0.05$) sand aggregate sizes than the control (Table 2.4). Between the temperatures of pyrolysis for each feedstock, the sand aggregate size fraction in soils with cotton biochar at 600°C was greater than at 400 and 500°C; soils with swine manure biochar at 600°C had greater sand aggregate size than at 500°C, but neither differed from swine manure biochar at 400°C. Soils with eucalyptus biochar at 400°C had greater sand aggregate size than at 500°C, but neither differed from soils with eucalyptus biochar at 600°C. Lastly, sand aggregate size in soils with filtercake biochar at 600°C was significantly greater than at 400°C, but not greater than at 500°C. Comparing silt+clay aggregate size between the treatments, there were no significant differences between the feedstocks or with the control, but there were differences between the temperatures of pyrolysis for each feedstock (Table 2.4). As with its sand aggregate size, cotton biochar at 600°C had greater silt+clay aggregate size than 400 and 500°C. Both swine manure and filtercake biochars showed decreasing silt+clay aggregate size with increasing temperature of pyrolysis, and soils with eucalyptus at 400°C had lower silt+clay aggregate size than at 500 and 600°C (Table 2.4).

Grain size distribution curves displayed heterogeneity in soils with biochars, ranging from high to low temperatures of pyrolysis in the order filtercake>eucalyptus>swine manure>cotton biochars.

The control soil had the least particle size heterogeneity (Figure 2.6). On examining the D10 and D90 of the grain size distribution, the fine sand (sand D10) did not differ between the feedstocks, whereas the coarse sand (sand D90) was greatest in soils with filtercake biochars and lowest in the control soil. Soils with cotton and filtercake biochars had similar silt+clay D10 and greater than the control, but silt+clay D90 did not vary. Comparing temperatures within the feedstocks, soils with cotton and filtercake biochars had higher sand D90 at 600°C than at 400°C, while silt+clay D90 was lower at 600°C than at 400°C for filtercake biochar, but higher at 600°C for cotton biochar (Table 2.4).

Table 2.4. Aggregate size and distribution of biochar-soil samples. Capital letters before values indicate significant differences between the feedstocks and lowercase letters indicate significant differences between the temperatures of pyrolysis for each feedstock (n = 3; Tukey or Games-Howell tests, $P < 0.05$, where variances were unequal).

Treatments		Sand aggregate size (>53µm)		Silt+clay aggregate size (<53µm)		Sand D10 (µm)		Sand D90 (µm)		Silt+clay D10 (µm)		Silt+clay D90 (µm)
Control	C	216.3±10.8	A	6.3±0.51	A	103.6±1.7	C	367.0±28.5	B	0.15±0.0	A	21.9±1.7
Cotton400	B	392.4±5.1 b	A	5.8±0.38 b	A	106.8±0.8 a	B	822.6±30.6 b	A	0.19±0.0 b	A	20.2±1.3 b
Cotton500		395.3±31.3 b		6.5±0.15 b		97.6±3.0 b		839.5±161.7 b		0.20±0.0 a		21.9±0.6 b
Cotton600		537.7±20.5 a		7.9±0.22 a		108.5±0.7 a		1694.8±76.4 a		0.19±0.0 b		26.1±0.7 a
Swine400	B	467.8±25.6 ab	A	7.1±0.43 a	A	106.6±0.7 b	B	1188.3±158.6 ab	AB	0.21±0.0 a	A	24.9±0.4 a
Swine500		345.1±5.6 b		4.8±0.15 b		104.2±2.0 b		636.0±11.7 b		0.14±0.0 b		17.4±1.5 b
Swine600		460.7±5.7 a		2.5±0.15 c		114.3±2.2 a		1240.1±24.6 a		0.13±0.0 b		8.8±1.5 c
Eucalyptus400	AB	604.8±64.5 a	A	3.5±0.1 b	A	115.1±0.9 a	AB	1629.9±201.1 a	B	0.16±0.0 a	A	11.2±1.4 b
Eucalyptus500		402.8±27.1 b		6.7±0.3 a		105.4±2.6 b		859.3±100.1 b		0.15±0.0 b		24.7±0.2 a
Eucalyptus600		450.8±31.5 ab		6.3±0.6 a		106.3±2.0 b		1179.2±166.6 ab		0.19±0.0 ab		20.0±0.7 a
Filtercake400	A	481.5±64.8 b	A	9.2±0.3 a	A	129.6±25.0 ab	A	1174.3±61.9 b	A	0.20±0.0 a	A	30.9±0.6 a
Filtercake500		775.0±4.4 ab		6.4±0.2 b		117.4±0.5 a		2589.0±103.6 a		0.20±0.0 a		22.8±0.6 b
Filtercake600		932.5±58.4 a		5.9±0.3 c		100.0±2.8 b		2829.3±8.9 a		0.19±0.0 a		21.6±1.2 b

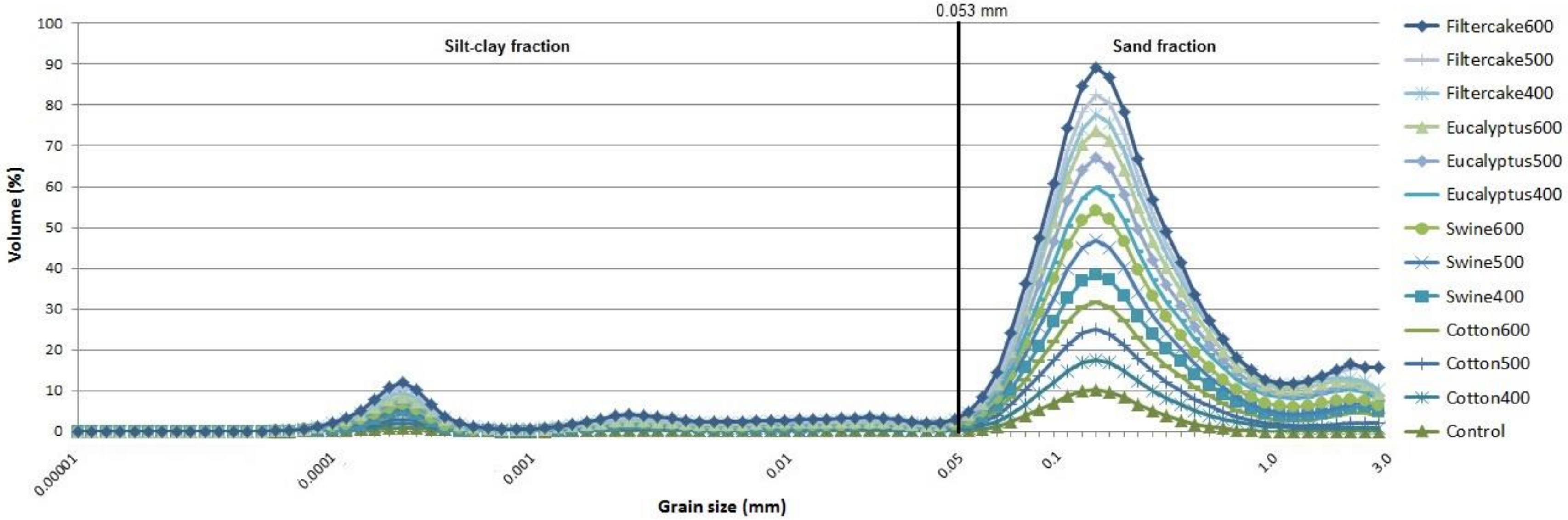

Figure 2.6. Grain size distribution curves of soils mixed with biochars compared to the unamended soil (control).

2.3.5 Principal component analysis

The PCA of soil and chemical properties showed several groupings for each of the four biochar feedstocks (Figure 2.7). Cotton biochars had three groupings: Ca, dry above and belowground biomass, Cu, Zn, and silt+clay D10 (Group 1), silt+clay D90, sand D10, EC, silt+clay aggregate size, sand D90, θ, and pH (Group 2), and AWC by itself (Group 3). The cotton biochars at 400°C clustered near Group 1, while the 500°C biochars clustered closely to Group 3 and the 600°C biochars by Group 2 (Figure 2.7A). For the swine manure biochars, the PCA showed four groupings: θ and EC (Group 1), silt+clay D90 and sand D10 (Group 2), sand aggregate size, Ca, sand D90, and silt+clay aggregate size, and dry above and belowground biomass (Group 3), with pH standing alone (Group 4). Swine manure biochars at 400°C clustered closely to Group 3, the 500°C biochars near Group 4, and the 600°C biochars were nearest to Group 1 (Figure 2.7B). For eucalyptus biochars, three groupings stood out: sand D10 and silt+clay D10 (Group 1), Ca, Fe, and Mn (Group 2), sand D90 and soil total C, pH, dry aboveground biomass, and sand aggregate size (Group 3). Eucalyptus biochars at 400°C clustered closely to Group 1, the 500°C biochars near Group 2, and the 600°C biochars near Group 4 (Figure 2.7C). Lastly, the PCA for filtercake biochars showed two main groupings: sand D90 and EC (Group 1) and bulk density, pH, Mn, Fe, Ca, sand D10, silt+clay D90, and dry above and belowground biomass (Group 2). Filtercake biochars at 400°C clustered closely to Group 1, while the 500°C and 600°C biochars clustered closely to the larger Group 2 (Figure 2.7D).

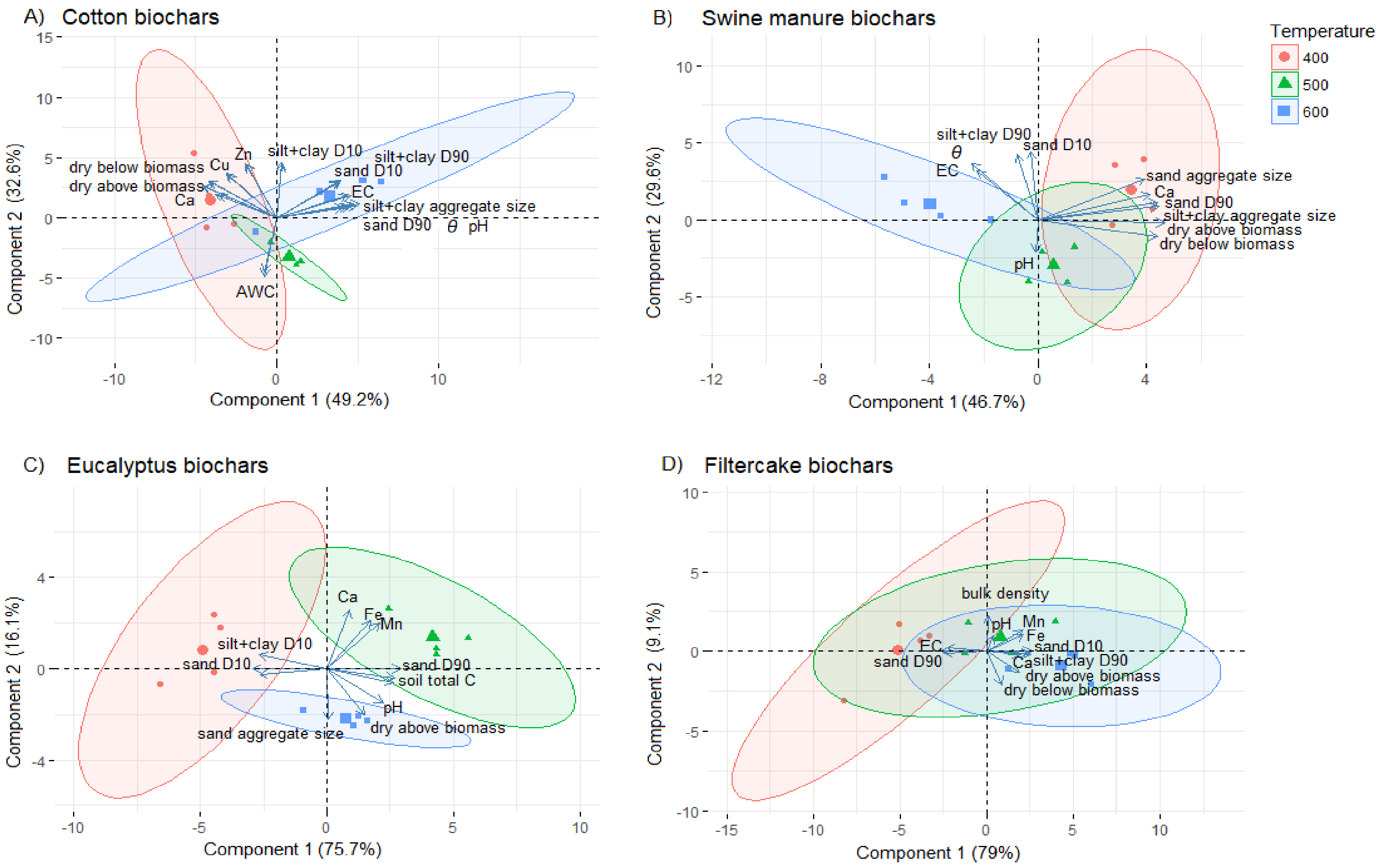

Figure 2.7. Principal component analysis (PCA) of chemical and physical properties of soils mixed with 12 biochars (4 feedstocks x 3 temperatures of pyrolysis, *n* = 4).

2.3.6 HYPROP

Observing the water curves of packed biochar-soil mixtures determined by the HYPROP®, cotton biochar at 400°C had the highest saturated water content (θ_s), followed by eucalyptus biochar at 600°C and the control soil (no biochar). All other biochar mixtures had lower θ_s than the control (Table 2.5).

Table 2.5. Parameter values for water content (θ) and K_s and fit quality of the model measured by root mean square error (RMSE) for each biochar-soil mixture treatment (HYPROP data). θ_s = saturated (wet soil) θ; θ_r = residual (dry soil) θ

	θ			K_s	
Treatment	θ_s (cm^3 cm^{-3})	θ_r (cm^3 cm^{-3})	RMSE$_\theta$	K_s (cm d^{-1})	RMSE$_{logK}$
Control	0.49	0.12	0.01	12.0	0.12
Cotton400	0.57	0.27	0.01	4.31	0.07
Cotton600	0.44	0.17	0.01	0.98	0.13
Swine400	0.46	0.16	0.01	1.22	0.09
Swine600	0.45	0.16	0.01	1.86	0.08
Eucalyptus400	0.41	0.16	0.01	0.70	0.13
Eucalyptus600	0.50	0.18	0.01	7.47	0.04
Filtercake400	0.46	0.16	0.01	2.23	0.06
Filtercake600	0.44	0.17	0.01	2.74	0.06

The hydraulic conductivity, *K*, of all the biochar-soil mixtures was higher than that of the control soil with no biochar (Figure 2.7). The control soil had the highest K_s (12.0 cm d^{-1}), suggesting water flowed easily through the soil and was poorly retained in soil pores. Eucalyptus biochar at 600°C (7.5 cm d^{-1}) and cotton biochar at 400°C (4.3 cm d^{-1}) also had high K_s; eucalyptus biochar at 400°C and cotton biochar at 600°C, however, had much lower K_s. All biochar-soil mixtures except for those with cotton biochar had higher K_s at 600°C than at 400°C (Table 2.5).

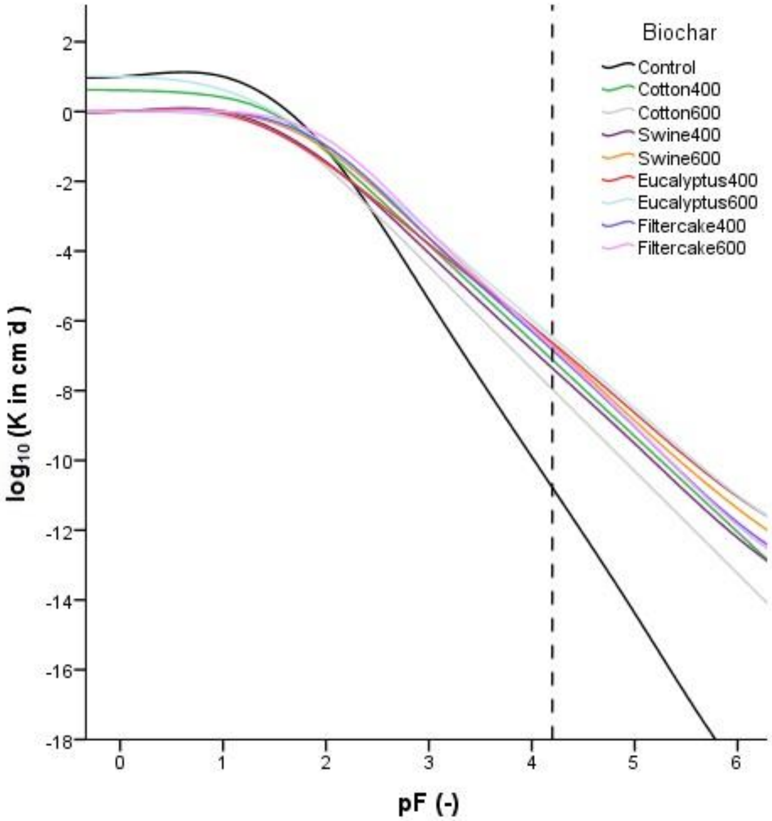

Figure 2.8. Hydraulic conductivity curves for packed biochar-soil mixtures, low and high temperatures of pyrolysis (HYPROP data; *n* = 1)

2.4 Discussion

2.4.1 Effect of biochar feedstock on plant biomass

The results of the greenhouse experiment suggest that water retention and nutrient levels, as reflected by EC, influenced plant biomass in biochar treatments. Filtercake and eucalyptus biochars did not significantly alter θ, EC, and AWC in soils compared to the control and maintained high maize biomass, filtercake biochar at 600 °C even leading to a 33% increase in maize biomass compared to the control (Figure 1; Appendix Figure B.1). In contrast, the high θ, EC, and AWC in soils with cotton and swine manure biochars led to lower maize biomass, as noted by the negative correlations (Table 2.1). PCA also showed negative correlations between biomass and pH, θ, EC, and AWC for cotton biochars, particularly for the 600°C biochars (Figure 2.7A) and for swine manure biochars, positive correlations between biomass and Ca particularly for the 400°C biochars (Figure 2.7B). The swine manure biochars at 600°C were closest to the positive correlation between θ and EC (Figure 2.7B), reflecting the high mean θ and EC levels in these treatments (Figure 2.3).

When added to the soil, biochar has the potential to increase pores in the 30 to 0.3 nm diameter range (Verheijen et al., 2009), but the pore size range for AWC is 0.2 to 30µm diameter (Hardie et al., 2014). In soils with cotton and swine manure biochars, the biochars may have increased soil porosity in the AWC pore range, leading to high AWC and nutrient content that was probably too high for the plants, essentially drowning them (i.e. causing hypoxia). The opposite may have also occurred, where chemical conditions caused more water to be bound to the biochar surface, increasing water volume in the biochar-soil mixture, but the water was immobile and thus unavailable for plant uptake or solute transport (Masiello et al., 2015). EC can indicate salinity, which refers to "the presence of major dissolved inorganic soil solutes in the soil aqueous phase" (p.17, Corwin and Lesch, 2005), including nutrients (or salinity ions) such as Na^+, K^+, Ca^{+2}, Mg^{+2}, SO_4^{-2} and NO_3^-. EC can be used then to determine crop yield potential. High salinity, however, can affect soil osmotic potential, reducing plants' ability to uptake water, as well as disrupt plants' nutritional balance (Corwin and Lesch, 2005). Biochar macropores in particular can discourage root hair growth into them because they can be saturated with immobile, anoxic water with high concentrations of salinity ions or phytotoxic organic compounds (Kammann and Graber, 2015). Thus despite having high θ, EC, and AWC, maize plants in soils with cotton and swine manure biochars may have been unable to uptake water and nutrients, limiting their growth particularly at higher temperatures of pyrolysis.

Maize has a high tolerance to salinity at ECs around 1.7 dS m^{-1}, 50% tolerance at 5.9 dS m^{-1}, and zero tolerance at 10 dS m^{-1} (Ayers and Westcot, 1994). Cotton and swine manure biochar treatments had EC levels between 4 and 8 dS m^{-1}, levels at which maize yield potential declines (Ayers and Westcot, 1994). In contrast, soils with eucalyptus and in particular filtercake biochars had ECs around 1 dS m^{-1}, likely allowing for free movement of water, soil aeration, and plant nutrient uptake. In addition, both eucalyptus and filtercake biochars contributed to high soil Ca levels, which were positively correlated with higher dry aboveground biomass (Appendix A.1B). Aboveground biomass also had positive correlations with soil Mn and Fe levels and for filtercake biochars, a negative correlation with EC as indicated by the PCA (Figures 2.7C and D). Major et al. (2010) noted that maize growth declined in a savannah Oxisol with low Ca and Mg levels; lower soil Ca levels in cotton and swine manure biochar treatments (Figure 2.2E) may similarly have also limited maize growth in our study. PCA for cotton biochars, in particular, showed a negative correlation between biomass and Ca (Figure 2.7A). These differences in maize biomass may thus be related to nutrient content, but are more likely related to salinity levels affecting net osmotic potential on total soil water potential.

2.4.2 Biochar contribution to soil water retention

Water retention and nutrient absorption by biochar is related to its high porosity (Major et al., 2009; Sorrenti et al., 2016; Verheijen et al., 2009). Because of its high porosity, biochar has been shown to increase AWC, as well as plant unavailable water at the permanent wilting point (Abel et al., 2013), mainly due to the high amount of micropores (Hardie et al., 2014). Biochar's porosity is determined both by the feedstock (which contributes mostly to macropores) and to the temperature of pyrolysis (which contributes to micropores and nanopores) (Brown et al., 2015; Uzoma et al., 2011b). While soil macropores (> 80µm) allow for rapid water flow and soil mesopores (between 30 and 80µm) allow movement from wet to dry areas, soil micropores (< 30µm) retain water (Major et al., 2009). In biochar, residual macropores (1 to 100µm) are formed from plant cellular structure and are believed to contribute to biochar pore volume, while pyrogenic nanopores (< 2 nm) are formed during pyrolysis and contribute mostly to biochar surface area (Gray et al., 2014). In sandy soils, biochar can behave like clay particles, holding large amounts of water which may be accessible to plants. High porosity is related to high surface area, which allows for greater nutrient adsorption. Biochar surface area usually increases with temperature of pyrolysis (Major et al., 2009), as was the case for our biochars (Table 2.3), where total surface area and micropore volume increased as temperatures increased from 400 to 600°C.

When our biochars were mixed with soil, however, the effect of biochar temperature of pyrolysis on water and nutrient retention was less than differences due to feedstocks. This is in agreement with Jeffery et al. (2015b) who did not observe either a biochar effect on water retention nor differences between the biochars at 400 and 600°C, despite the biochar 600°C having higher porosity. Although temperature of pyrolysis is considered the most important factor determining physical changes of biochar, feedstock type determines the temperature range under which changes occur (Downie et al., 2009). Typically, the pore structure of a biochar resembles the cellular structure of the feedstock in wood or plant-based biochars. The organic and inorganic components (e.g. ash) can affect biochar structure as temperatures of pyrolysis increase, increasing decomposition and/or reacting with the C lattice structure (Chia et al., 2015). Yet, on examining scanning electron microscopy (SEM) images of our 12 biochars (Appendix Figure

C.1 and C.2), pore sizes do not appear to differ between the temperatures of pyrolysis (400, 500 and 600°C) for each feedstock. Comparing the feedstocks, however, micropores in cotton and eucalyptus biochars appear stacked and longitudinal, while micropores in swine manure biochar appear irregularly, like pores in a sponge. Unlike the other biochars, micropores in filtercake biochar are barely visible, corroborated by its low micropore volume (Table 2.3). Eykelbosh et al. (2014) likewise observed large irregular macropores with few micropores in SEM images of their filtercake biochar produced at 575°C.

Filtercake biochar's low micropore volume, but high total pore and mesopore volumes and surface areas, suggests that it contained more meso- and macropores that allowed for sufficient drainage in the soil, and therefore θ and AWC in soils with filtercake biochar did not differ from the control. Eucalyptus biochar had high total surface area, but low total pore volume. Biochars made from wood often have large macropores (~10μm) (Sun et al., 2012), but the low total pore volume of the eucalyptus biochars may suggest it contained smaller macropores or mesopores. Lee et al. (2013) observed high surface area for palm kernel shell biochar (hard shell residue from crushed palm nuts heated to 500°C) due to the presence of mesopores. Although mesopores for only eucalyptus biochar at 500°C were measurable in our analysis, more small mesopores rather than micropores in eucalyptus biochar could have also promoted drainage so that θ in soils with eucalyptus biochar did not differ from filtercake biochar treatments or the control.

The high θ in soils with cotton and swine manure biochars would suggest that these biochars had high porosity and surface area that contributed to high soil water retention. Swine manure biochars had high total surface area similar to filtercake biochars, but they contained lower mesopore surface area and volume and higher micropore surface area and volume than filtercake biochars. Less mesopores and more micropores may have contributed to greater water retention in soils mixed with swine manure biochars. The cotton biochars, however, surprisingly had the lowest total surface area and micropore volume of all the biochar feedstocks, although its micropore surface area was greater than its total surface area and mesopore area (Table 2.3). In the literature it has been noted that the hydrologic properties of biochars cannot be entirely determined before adding it to the soil. A biochar can have different effects on the soil hydrology depending on the soil, so that the hydrology of the biochar-soil mixture is not directly related to the hydrology behaviour of biochar alone (Masiello et al., 2015). This appears to be the case for cotton biochar in our study which contributed to high soil water retention, despite the cotton biochars alone having similar or lower micropore surface area or volume compared to the other biochars (Table 2.3).

2.4.3 Biochar particle size and distribution

Particle size analysis of the biochars showed filtercake biochar had significantly lower mean particle size than the other biochars, which did not differ from each other (Table 2.3). In addition, filtercake biochar had lower D90 than the other biochars. The negative correlation between total pore volume and particle size of the biochars suggests that filtercake biochar’s high total pore volume was influenced by its low particle size and low coarse fraction distribution. Cotton biochars had low total pore volume, but large particle size. Swine manure and eucalyptus biochars also had large particle sizes compared to filtercake biochar, but higher total pore volume than cotton biochar (Table 2.3). Biochars can alter soil water dynamics by changing the size, shape and amount of pores between soil particles (Masiello et al., 2015). For cotton biochar in particular, its large particle size may have reduced some or all of these properties in the soil.

When mixed in soil, the biochars led to significantly greater sand aggregate sizes compared to the control, with soils with filtercake biochars having the highest mean sand aggregate size (Table 2.4), despite the biochar alone having a low coarse fraction (Table 2.3). Larger biochar particles might remain closer to the soil surface, while smaller particle size could allow the biochar to move further down the soil profile (Brodowski et al., 2007). Biochar added in one layer has been observed to reduce water loss in a sandy soil compared to biochar mixed uniformly, as the biochar layer can slow down water flow and evaporation (Zhang et al., 2016). Filtercake biochar particles may have settled more uniformly in the soil matrix with each watering, as noted by its high contribution to sand D90 and silt+clay D10, significantly greater than the control (Table 2.4) and possibly leading to greater heterogeneity in grain size distribution in the soil mixture (Figure 2.5). PCA found that both sand D10 and silt+clay D90 were positively correlated with aboveground biomass, while negatively correlated with sand D90 and silt+clay aggregate size (Figure 2.7) The greater heterogeneous distribution, high fine sand and coarse silt+clay fractions, and high sand aggregate sizes in soils with filtercake biochar (especially at 600°C compared to at 400°C) may have allowed for water to flow and drain more freely, thus providing low water retention, but without impacting plant growth. Eucalyptus biochar likewise contributed to a high sand D90 and silt+clay D10 similar to the control, and aboveground biomass was positively correlated with sand D90, soil total C, and pH (Figure 2.7). Both filtercake and eucalyptus biochar treatments had low soil water content, but both nevertheless had higher mean AWC than the control. Furthermore, the high Ca/Mg ratios in filtercake and eucalyptus biochar treatments (Appendix Figure A.2) suggest better soil structure, with higher ratios related to improved water infiltration (Hartz, 2007). Soils with greater Ca than Mg can improve soil aggregation by increasing flocculation, leading to less surface sealing and higher infiltration rates (Dontsova and Norton, 2001). PCAs for eucalyptus and especially filtercake biochars found positive correlations between Ca, Fe, and Mn (Figure 2.7). Metals such as Fe and Mn, along with lower pH, can contribute to the formation of organo-mineral or organo-metallic associations that decrease biochar mineralization, increasing biochar-C stability in the soil which may improve soil structure (Fang et al., 2013).

In contrast, soils with cotton and swine manure biochars had silt+clay D10 similar to soils with filtercake biochar, but both had lower sand D90. Separating miscanthus and wheat biochars into three particle size fractions (0–500 µm,500–1000 µm, and 1000–2000 µm) and mixing into a loamy sand, Glab et al. (2016) observed higher field capacity and AWC in soils with the 0–500 µm biochar fraction applied at 4% w/w. In the present study, the high fine silt+clay fraction in soils with cotton and swine manure biochars could have also led to their higher mean water retention and AWC (Figure 2.3 and 2.4) compared to the filtercake and eucalyptus biochars, as well as the control. Furthermore, larger biochar particles were visible on the soil surfaces of pots with cotton and eucalyptus biochars (e.g. the 600°C biochars, Appendix Figure D.1). In the case of cotton biochar, the possible formation of biochar layers within the soil combined with its low heterogeneous grain distribution (Figure 2.5) and high contribution to the fine silt+clay fraction may have exacerbated soil water retention.

2.4.4 Biochar effect on hydraulic conductivity

All biochar-soil mixtures in our study had higher K than the control soil (Figure 2.6), but lower K_s than the control (Table 2.5). This is consistent with other laboratory studies that reported decreased K_s in biochar-sandy soil mixtures (Barnes et al., 2014; Brockhoff et al., 2010; Githinji, 2014), perhaps due to increased tortuosity in the interstitial space between biochar and sand particles (Barnes et al., 2014). Thus biochars in our packed cores improved water flow (infiltration), but reduced drainage. Mixing biochar with sand can increase K_s due to biochar's greater porosity, particle size and bulk density, but decrease K_s in a clay-rich soil (Barnes et al. 2014). Although lower than cotton at 400°C and eucalyptus at 600°C, K_s values were higher in soils with filtercake biochar at both temperatures compared to the other biochars (Table 2.5), consistent with its high contribution to the soil sand fraction (Table 2.4). In addition, in unsaturated soils, K remained high for soils with filtercake and eucalyptus biochars at the permanent wilting point (pF 4.2), while K in soils with cotton at 400°C and 600°C, as well as swine manure at 400°C, was lower (Figure 2.6). This suggests that water moved more slowly in soils with the latter biochar feedstocks as they dried compared to the soils with filtercake and eucalyptus biochars.

The K_s for the high temperature biochars was greater than that for the low temperature biochars for all feedstocks except for cotton (Table 2.5). Considering biochar porosity increases with increasing temperature of pyrolysis (Brown et al., 2015), it would be expected that soils with higher temperature biochars would drain slower than soils with lower temperature biochars, but this did not seem to be the case for our biochar-soil packed cores. However, there are still limited studies on the effect of biochar on K and the physical and chemical controls on it are not yet well described. There are several factors that can influence K in biochar-soil mixtures, including biochar feedstock, biochar production, both soil and biochar particle shape, plant effects, and biochar effects on soil aggregation (Masiello et al., 2015). Indeed, Hardie et al. (2014), in a field experiment mixing acacia tree biochar at 550°C into a sandy loam, found no evidence of direct biochar effect on soil porosity, AWC, and soil water retention, while Glab et al. (2016) observed no change in K_s in biochar-sandy soil mixtures. Biochar hydrophobicity can be another factor, causing lower water-holding capacity and K (Masiello et al., 2015), but was not measured for our biochars. Nevertheless, *K* was higher in all biochar-soil mixtures compared to the control (Figure 2.6), suggesting the biochars had little hydrophobicity. Moreover, other studies (Herath et al., 2013; Kinney et al., 2012) have shown that biochar addition does not increase soil hydrophobicity and, if necessary, biochar hydrophobicity can be reduced by rinsing the biochar with water before application (Kinney et al., 2012). In our study, possible formation of biochar layers in soils with cotton biochar at 600°C (and perhaps eucalyptus at 400°C) as opposed to a more uniform distribution within the soil matrix could have reduced K_s (Table 2.5), as observed by Zhang et al. (2016) in a sandy soil column experiment. Both eucalyptus and cotton biochars at 600°C led to significantly lower soil bulk density compared to the control (Table 2.2), but this appears to have contributed to higher *K* only in soils with eucalyptus at 600°C and not with cotton biochar. Thus, the variety of factors to take into account when examining biochar-soil mixtures makes it difficult to generalize biochar's contribution to soil hydrology, as it remains very much soil- and biochar-specific.

2.5 Conclusions

Overall, the agricultural waste biochars assessed in this study showed potential for increasing water retention when mixed with a sandy soil compared to the soil alone. Water retention levels did not vary significantly between temperatures of pyrolysis as hypothesized, but varied between feedstock types. All biochar-soil mixtures had lower K_s than the control, suggesting they decreased water drainage in the sandy soil. Analysis of the porosity, surface areas, and SEMs of the biochars did not always coincide with the effect of the biochars mixed with soil, especially when comparing temperatures of pyrolysis. Yet, the properties of the biochars cannot always predict its effect in the soil, as many factors come into play including soil and biochar particle size and the presence of plants. This was especially evident in cotton and swine manure biochar treatments which showed potential to increase soil water retention and nutrient content more than unamended soils, but nevertheless had a negative effect on maize biomass. This was probably related to high salinity and their contribution to the fine silt+clay fraction in the soil, whereas filtercake biochar treatments, which had the highest mean maize biomass, contributed most to the coarse sand fraction. Filtercake biochar, particularly at 600°C, may have contributed more to soil structure and aeration, allowing for maize to grow well, while both cotton and swine manure biochars at either high or low temperatures of pyrolysis increased soil water retention the most. In summary, filtercake and eucalyptus biochars show potential for maintaining or even increasing plant growth due to improved soil moisture dynamics, while applying cotton and swine manure biochars at low levels could contribute to increased soil water resilience of Cerrado Arenosols. If applied at a suitable rate so as not to cause excessive water or nutrient content, these biochars could make a significant contribution to crop production in times of drought.

Chapter 3: Maize growth as influenced by agricultural waste biochars applied at varying rates in a Cerrado region (Brazil) Arenosol

3.1 Introduction

The state of Mato Grosso, Brazil encompasses a large portion of the Brazilian savanna biome, known as the Cerrado. Characterized by different grassland vegetation gradients including open field Cerrado, dense Cerrado, and open arboreal Cerrado (Maia et al., 2009), the biome is increasingly being transformed for agricultural use. The dominant soil type in the region is Ferralsol (FAO classification), but other soil types including Arenosols (sandy soils) are also being transformed for crops. Arenosols cover about 13% of the area of Mato Grosso, and are increasingly being converted to agriculture, particularly for growing maize (SEPLAN, 2008). In most of Mato Grosso, double cropping (planting two crops per year) has become a common practice in which one crop is grown during the rainy season (e.g. soybean – usually grown from October to February) and another at the end of the rainy season (e.g. maize or cotton – usually grown from February to July). The maize crop, when planted after the soybean harvest in February, is known as *safrinha* or *segunda safra* (second crop), and accounts for 98% of all the maize grown in Mato Grosso (IBGE, 2016). The importance of maize *segunda safra* has risen significantly in the last 10 years, with total maize production in Mato Grosso exceeding 20 Mt y^{-1} in recent years (IBGE, 2016). Yet, since it is planted at end of the rainy season and harvested in the dry season, the maize *segunda safra* depends largely on residual soil moisture and is thus vulnerable to changes in precipitation patterns (Cruz et al., 2010) due to global climate change. In addition, the region’s high precipitation rates (mostly during the rainy season) and base cation leaching lead to the development of acidic soils, which results in soils with low cation exchange capacity (CEC), low base saturation levels and low water-holding capacity (Costa et al., 2013; Fageria, 2001).

A wide-spread management practice used in the area is liming, which helps improve nutrient deficiencies (e.g. calcium and magnesium) in these soils (Lopes, 1996). However, other management practices can be used in combination to help increase productivity of sandy Cerrado Arenosols. The potential of biochar (charcoal derived from waste biomass by pyrolysis) to increase soil water and nutrient retention has been observed under some conditions (Abel et al., 2013; Lehmann, 2007; Sohi et al., 2009; Spokas et al., 2012), and so its amendment to sandy soils could potentially be beneficial in this system. Biochars with high pH can also reduce some liming requirements in acidic soils (Biederman and Harpole, 2013). Furthermore, since biochar can be made from any organic waste (e.g. crop residue, papermill sludge, sewage sludge, animal manure), transforming crop residue from farms into biochar can provide an alternative way to utilize these waste materials (Lehmann and Joseph, 2015b). In this study, four feedstock materials were chosen to produce biochar based on their environmental impact and the agricultural aptitude of Mato Grosso: cotton husks, sugarcane filtercake (a residue left over from filtration of sugarcane juice after clarification), swine manure, and eucalyptus residue. Filtercake, cotton husks and swine manure all currently pose disposal challenges and environmental contamination issues. While eucalyptus residues are currently used for bio-energy, this material was included to facilitate comparison to a woody feedstock, as woody feedstocks are commonly used in biochar studies.

Using these organic wastes readily found in Mato Grosso as potential biochar feedstocks would substantially reduce their biomass volume and result in biochars comprised of a more stable organic material that minimizes nutrient loss, and significant for the case of swine manure, is free of pathogens following pyrolysis (Lehmann and Joseph, 2009). In this way, these organic wastes become safe to apply to the soil and may contribute to improved soil fertility and plant health. Less understood is the effect of biochar on plant physiological characteristics, such as photosynthesis rate, which is a key indicator of plant fitness (Xu et al., 2015). Depending on the feedstock, biochar can increase soil nutrient content and potentially boost plant growth (Hass et al., 2012; Jeffery et al., 2011; Spokas et al., 2012; Uzoma et al., 2011a). However, recent studies have found biochar additions can improve (Akhtar et al., 2014), reduce (Kammann et al., 2011), or not affect (Alburquerque et al., 2013) photosynthesis. To address these uncertainties, our study had the following objectives: 1) to determine the effect of biochar type and application rate on maize biomass production and physiological characteristics, and 2) to evaluate the impact of biochar applications on resulting nutrient levels in the soil. As the intent of the study was to test the role of different biochars to observe if there would be negative interactions or positive synergies, plants were not stressed and were not exposed to limiting conditions in terms of water and nutrients. It was hypothesized that swine manure biochar would increase soil nutrient levels and lead to greater maize biomass compared to the other biochar feedstocks since animal manures are often rich in nutrients (Chen et al., 2008) compared to plant feedstocks.

3.2 Materials and methods

3.2.1 Soil collection and biochar production

Soils from the top 0-20 cm layer were collected from an agricultural field located within the farm Fazenda Água Azul (15°13'55.2"S, 54°57'43,4"W) managed by the agribusiness Grupo Bom Futuro, 178 km northwest of the state capital of Cuiabá in Mato Grosso, Brazil, an area within the Cerrado. The climate is described as tropical, hot semi-humid with average monthly temperatures above 18°C year-round. The dry season lasts 4 to 5 months, beginning around

May/June to September/October (IBGE, 2014). Precipitation rates are between 1500 and 2250 mm year^{-1} (Maia et al., 2009). The soil collected was classified as an Arenosol (FAO soil classification), with a sandy texture (91% sand, 4% silt, 5% clay). Carbon (C) and nitrogen (N) levels in the soil were 0.7 % C and 0.08 % N as determined by elemental analysis (628 Series CHN Analyzer, LECO Corp., St. Joseph, MI). The average pH in water (pH_{water}) was 5.8 and average CEC was 5.3 cmol$_c$ kg^{-1}, with a bulk density of 1.6 g cm^{-3}. Over the last 10 years, the crops sown on the study site included soybean, sorghum, maize, and cotton, with the latter two crops grown in rotation with soy for the last three years (Afonso Campos da Silva, Grupo Bom Futuro, personal communication, 2014). Biochars derived from four feedstocks (cotton husks, eucalyptus residue, sugarcane filtercake, swine manure) were pyrolised at 400°C (SPPT Ltda., Mogi Morim, São Paulo, Brazil), then crushed and sieved to <2 mm in order to have similar biochar particle sizes between the different feedstocks and similar to soil particle size. Chemical properties of the soil, as well as the biochars used, are further described in Table 3.1.

Table 3.1. Chemical properties of an Arenosol and biochars made from agricultural waste feedstocks (cotton husks, swine manure, eucalyptus sawdust, sugarcane filtercake) pyrolyzed at 400°C. Values are means (*n* = 3)± 1 SE. CEC = cation exchange capacity.

	Soil	**Cotton400**	**Swine400**	**Eucalyptus400**	**Filtercake400**
Total C (%)	0.7±0.03	54.8±2.0	25.4±9.6	54.6±7.3	28.1±0.5
Total N (%)	0.08±0.02	3.1±0.1	2.8±0.8	1.6±0.01	2.9±0.01
pH_{water}	5.8±0.1	10±0.6	9.2±0.3	7.7±0.03	8.6±0.6
CEC (cmol$_c$ kg^{-1})	5.3±0.3	49.1±3.8	28.7±1.1	3.7±0.2	3.9±0.3
P (mg kg^{-1})	82.4±23.0	3700±800	6500±400	200±40	1300±100
K (mg kg^{-1})	43.3±4.1	17,300±1200	9100±700	700±70	600±100
Ca (cmol$_c$ kg^{-1})	2.2±0.2	2.0±0.2	2.9±0.1	1.5±0.06	1.9±0.1

	Soil	Cotton400	Swine400	Eucalyptus400	Filtercake400
Mg (cmol$_c$ kg^{-1})	0.7±0.04	2.7±0.5	2.7±0.6	0.3±0.01	0.4±0.02
S (mg kg^{-1})	7.6±0.6	120.1±4.5	50.3±16.8	9.5±1.0	10.4±1.1
Cu (mg kg^{-1})	0.97±0.3	5.0±1.3	12.1±4.5	1.0±0.1	2.4±0.4
Fe (mg kg^{-1})	79.0±4.5	35.7±0.2	12.7±1.8	170±13.2	266±6.0
Mn (mg kg^{-1})	9.8±1.7	27.4±4.0	41.9±7.3	9.1±1.6	84.9±1.0
B (mg kg^{-1})	0.5±0.03	5.4±2.5	2.2±0.7	0.6±0.1	0.8±0.1
Zn (mg kg^{-1})	2.8±0.9	31.0±5.3	21.57±7.7	2.4±1.0	17.4±0.3

3.2.2 Experimental set-up

In a greenhouse located at the Federal University of Mato Grosso, Cuiabá campus, the biochars were added to 8 kg of an Arenosol soil at 1, 2, 3, and 4% on a dry weight basis (equivalent to 16, 32, 48, 64 t ha^{-1}) and mixed in 9L pots by hand. The treatments were thus four biochar types x four application rates, replicated four times plus four controls, for a total of 68 pots. Water was then added to 60% water filled pore space (WFPS) equivalence and the pots divided into four blocks (one block per greenhouse bench) for a randomized complete block design. Temperature in the greenhouse was set to 28±2°C, near temperatures during which the *safrinha* is grown from January to June (INPE, 2012).

Since fertilizer applications are a standard management practice in the region, fertilizer was added to the pots corresponding to the amount each maize plant requires at the rate of 150 kg NPK+S, 150 kg KCl and 200 kg urea for 60,000 plants/hectare in the field (Afonso Campos da Silva, Grupo Bom Futuro, personal communication, 2014). After one week, 2.5 g of crushed NPK+S (12-46-0 + 7) was mixed into soil in the center of each pot. Four maize seeds (hybrid seed, Dekalb) were planted in all pots around the center to allow roots to spread out evenly and thinned down to one plant per pot after ten days. After 20 days, 2.5 g of crushed KCl and 1.2 g of urea diluted in 50 mL of water were added to each pot. Seven days later, another 1.2 g of diluted urea was added, followed by a third application of 1.2 g another 7 days later, replicating nutrient management strategies typical in the region. Soil moisture was maintained at 60%WFPS by checking soil moisture three times per week using a GS3 sensor (Decagon Devices, Inc., Pullman, WA, USA) inserted into the top 10 cm of soil. Soil electrical conductivity (EC) was recorded at the same time from GS3 sensor output. All plants, with and without biochar, thus received adequate amounts of water, fertilizer, and growing conditions. Pots were rotated within blocks to decrease effect of any temperature and light differences within the greenhouse space.

3.2.3 Plant physiological measurements

Plant physiological characteristics were measured halfway through the experiment (day 20) and on the last day (day 42) to observe biochar effects on plant processes. Physiological measurements were made in the morning (between 8 am and 1 pm) for a subset of treatments, with the 1% biochar application rate chosen as plant sizes at this dose were most similar for all biochar types. Measurements were made on four replicates per feedstock (one replicate per block). Before starting the measurements, a test was carried out to confirm there were no physiological variations due to leaf senescence. Four leaves in good health (i.e., no signs of chlorosis) located between the first node above the soil and the topmost leaf were selected for one plant per treatment. Measuring at different points of each leaf, there was reduced gas exchange in the lower leaves, but no significant differences in physiological parameters (e.g. photosynthesis, transpiration) between the leaves ($P > 0.05$). Thus, the third, fully-expanded leaf of each plant (counting from the base of the plant) was used for measurements described below.

Gas exchange measurements were made with a portable photosynthesis system LI-6400XT (LI-COR Inc., Lincoln, NE, US). An area located in the middle third, 2 cm from the leaf edge, of each leaf was subjected to a photon flux of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to guarantee light saturation for photosynthesis. A block temperature of 28 °C, a reference CO_2 air concentration fixed at 400 $\mu mol\ mol^{-1}$ and a reference relative humidity of 60% were used to minimize stomatal heterogeneity. Net photosynthetic rate (P_n, $\mu mol(CO_2) \cdot m^{-2} \cdot s^{-1}$), transpiration rate (E, $mmol(H_2O) \cdot m^{-2} \cdot s^{-1}$)) and stomatal conductance (g_s, $mol(H_2O) \cdot m^{-2} \cdot s^{-1}$) were calculated by the LI-6400XT data analysis program. Intrinsic water use efficiency (WUE_s, $\mu mol(CO_2) \cdot mol^{-1}$ (H_2O) was calculated as P_n/g_s (Dalmagro et al., 2016) and water use efficiency of photosynthesis (WUE_t, $\mu mol(CO_2) \cdot mmol^{-1}$ (H_2O)) as P_n/E (Kammann et al., 2011). Dark respiration rate (R_d, $\mu mol \cdot m^{-2} \cdot s^{-1}$) was measured after switching off the light for about 20min.

Water use efficiency of productivity (WUE_{Prod}, g L^{-1}) was calculated as total dry aboveground biomass at the end of the experiment per total water consumed per pot (Kammann et al., 2011). Total water consumed (L) was the sum (Σ) of water added to the pots beginning two days after water was first added to 60% WFPS until the end of the experiment. Leaf area (LA, mm^2) of the third, fully-expanded leaf of each plant was measured immediately after harvest on day 42 using a leaf area meter (CI-202, CID, Inc., Camas, WA, USA). LA of the entire plant was also determined. The third leaf tissue was subsequently ground using a ball mill (Mini Mill Pulverisette 23, Fritsch GmbH, Oberstein, Germany) and analysed for leaf total C and N on a CHN analyzer (628 Series, LECO Corp., St. Joseph, MI). Specific leaf area (SLA) was calculated as the ratio between total LA and total dry leaf biomass. Leaf nitrogen use efficiency (NUE_{Prod}) was calculated as the ratio between total dry aboveground biomass and mg of leaf N (g mg^{-1} leaf N) (Kammann et al., 2011). Additional properties calculated based on readings at the end of the experiment and the total leaf area were: plant photosynthesis (P_{plant}, $\mu mol(CO_2) \cdot m^{-2} \cdot s^{-1} \cdot mm^{-2}$), aboveground plant respiration (R_{plant}, $\mu mol \cdot m^{-2} \cdot s^{-1} \cdot mm^{-2}$), plant stomatal conductivity

(g_{plant}, mol(H_2O)·m^{-2}·s^{-1}·mm^{-2}), and plant aboveground transpiration (E_{plant}, mmol(H_2O)·m^{-2}·s^{-1}·mm^{-2}) (Kammann et al., 2011). Photosynthetic nitrogen use efficiency ($PNUE_{plant}$) was calculated as P_{plant} per mg of leaf N (µmol(CO_2)·g N^{-1} ·s^{-1}) (Xu et al., 2015).

3.2.4 Plant and soil analysis

On day 42, maize plants were cut at the base and the following plant physical characteristics were immediately quantified: number of leaves, stem diameter, plant height to the tip of the top leaf, and total fresh biomass. Plant leaves from the 1% biochar application rate were tested for physiological characteristics. Leaves were first cut from the stems to measure total LA and then weighed fresh. Plant biomass was subsequently dried for 48h at 60°C and the dry weight recorded. Dried weight of the leaves for the 1% dose plants was recorded separately before combining with the stem for total dry aboveground biomass. Pots were destructively sampled to collect all fresh root biomass and soil samples. Soil samples were analyzed for macronutrient (available P, K^+, Ca^{2+}, Mg^{2+}, and S) and micronutrient (Zn, Cu, Fe, Mn, B) availability, pH_{water} (1:2.5), and CEC according to the standard soil methodologies used by the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, 2009) as described in Eykelbosh et al. (2014). Soil total C and N was analyzed on a CHN Analyzer (628 Series, LECO Corp., St. Joseph, MI).

3.2.5 Statistical analyses

All dependent variables were tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene’s tests, respectively, prior to statistical analysis. The effects of biochar feedstock and biochar application doses on maize biomass, soil properties, and macro- and micronutrients were determined by multivariate ANOVA (MANOVA) using IBM® SPSS® Statistics (Version 23, SPSS Inc., Chicago, USA). Where treatments were significant, a post-hoc Tukey test ($P < 0.05$) was used to compare means between feedstocks and doses when variances were equal, and a post-hoc Games-Howell test when variances were not equal. Pearson correlation coefficients and linear regression were determined between above and belowground biomass and soil properties and nutrients.

For the physiological characteristics, a repeated measures ANOVA was carried out for P_n, g_s, R_d, E, WUE_s, and WUE_t measured on day 20 and day 42, with Treatment the between-subject factor and Time as the within-subject factor. A MANOVA was carried out for plant physiological properties based on total leaf area or total aboveground dry biomass: P_{plant}, R_{plant}, g_{plant}, E_{plant}, ∑water consumed, WUE_{Prod}, NUE_{Prod}, and $PNUE_{plant}$. A MANOVA was also performed for plant physical properties determined at the end of the experiment: total LA, stem diameter, number of leaves, plant height, total fresh leaf biomass, total dry leaf biomass, and SLA, as well as leaf C:N ratio. Correlations and regressions were also performed between plant and soil variables. Principal component analysis (PCA) was carried out on soil and plant properties of the 1% dose biochar treatments and the control using the FactoMineR package (Le et al., 2008) in R (version 3.3.1). Values are presented in text and graphs as means ± 1 standard error (SE).

3.3 Results

3.3.1 Plant biomass and soil properties

Biochar amendments had a significant effect on dry above and belowground maize biomass (Figure 3.1), with dry above and belowground biomass positively correlated (R = 0.88, $P < 0.01$). Between the feedstocks, maize biomass for soil amended with filtercake and eucalyptus biochars at all application rates had significantly greater mean dry aboveground biomass than maize from soils with cotton and swine manure biochars at all rates ($P < 0.05$, Figure 3.1A). In addition, filtercake biochars had higher mean aboveground biomass at three application rates (1% dose: 18.1±0.8, 2% dose: 16.4±1.3, and 4% dose: 18.1±5.6) compared to the control

(15.1±1.7), though these were not significantly different based on a Tukey test. For eucalyptus and filtercake biochars, there were no significant differences between the application rates, but for cotton biochar, biomass decreased significantly with increasing application rate, while for swine manure biochar the difference was only significant between the 1% dose and the other three doses. Belowground biomass exhibited similar patterns as the aboveground biomass, with maize root biomass from soils with eucalyptus and filtercake biochars not significantly different from each other or the control, but greater than root biomass from cotton and swine manure biochars (Figure 3.1B).

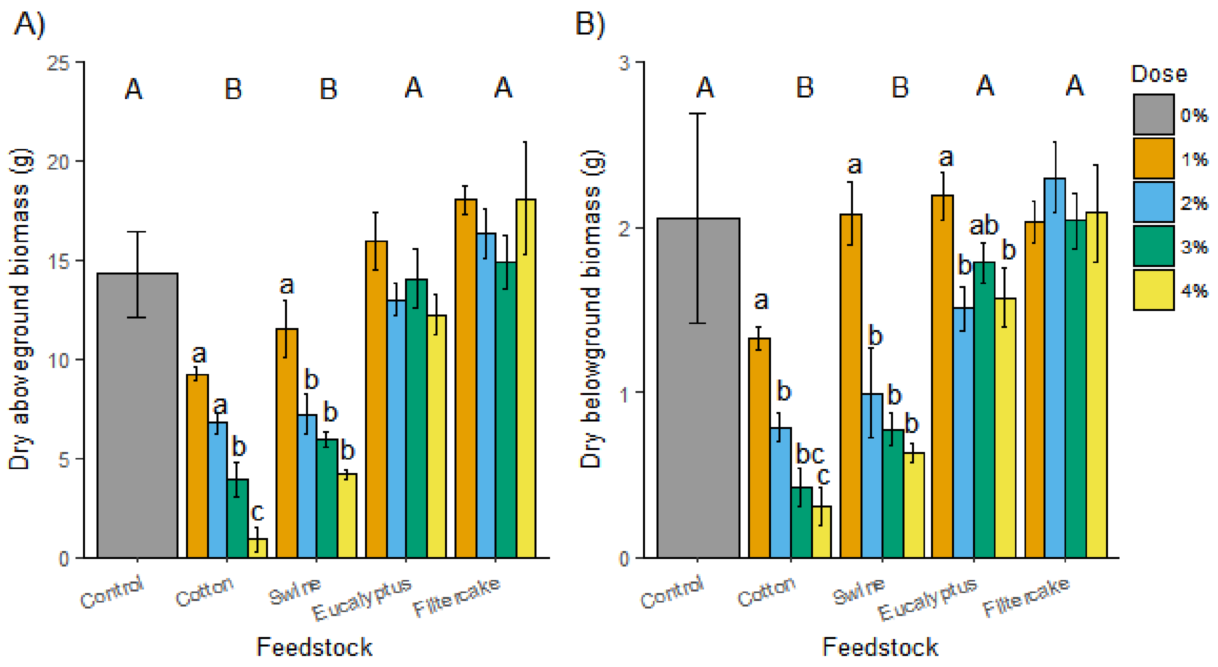

Figure 3.1. Mean dry aboveground biomass (A) and belowground biomass (B) by biochar feedstock and doses after 6 weeks (*n* = 4). Capital letters indicate significant differences between the feedstocks and lowercase letters indicate significant differences between doses for each feedstock (Tukey test; *P*<0.05).

pH levels for cotton and swine manure biochars increased with increasing dose, while varying less for eucalyptus and filtercake biochars (Table 3.2). Pairing soil pH levels with dry aboveground biomass showed a significant negative correlation (R = -0.70, $P < 0.001$) (Appendix Figure E.1A), which was the same for belowground biomass (R = -0.70). Soil CEC increased significantly ($P < 0.05$) only in soils with swine manure biochars compared to the control (6.1±0.5 $\text{cmol}_c\ \text{kg}^{-1}$). Comparing the biochars, soils with swine manure biochar (8.5±0.4 $\text{cmol}_c\ \text{kg}^{-1}$) had greater CEC levels than with eucalyptus biochar (6.8±0.3 $\text{cmol}_c\ \text{kg}^{-1}$), but neither differed from soils with cotton (7.5±0.4 $\text{cmol}_c\ \text{kg}^{-1}$) and filtercake biochars (7.1±0.3 $\text{cmol}_c\ \text{kg}^{-1}$) (Figure 3.2). CEC levels were poorly correlated with aboveground dry biomass (R = -0.32, $P < 0.01$) (Appendix Figure E.1B).

Table 3.2. pH_{water} levels of soils with cotton, swine manure, eucalyptus, and filtercake biochars at 1-4% doses after 6 weeks. Values are means ± 1 SE. Lowercase letters indicate significant differences between doses at $P < 0.05$ (Tukey test). Capital letters next to feedstocks indicate significant differences ($P < 0.05$) between the feedstocks and asterisk (*) indicates biochars that significantly ($P < 0.01$) increased soil pH compared to the control soil pH (4.9).

	pH_{water}			
Dose	**Cotton A***	**Swine manure A***	**Eucalyptus C**	**Filtercake B***
1%	5.8±0.1 c	6.1±0.1 d	4.9±0.1 b	5.3±0.1 b
2%	6.6a±0.1 b	6.6±0.1 c	5.2±0.1 ab	5.7±0.1 b
3%	7.1±0.4 ab	6.9±0.1 b	5.1±0.1 ab	5.8±0.2 b
4%	7.5±0.3 a	7.2±0.1 a	5.3±0.1 a	6.4±0.1 a

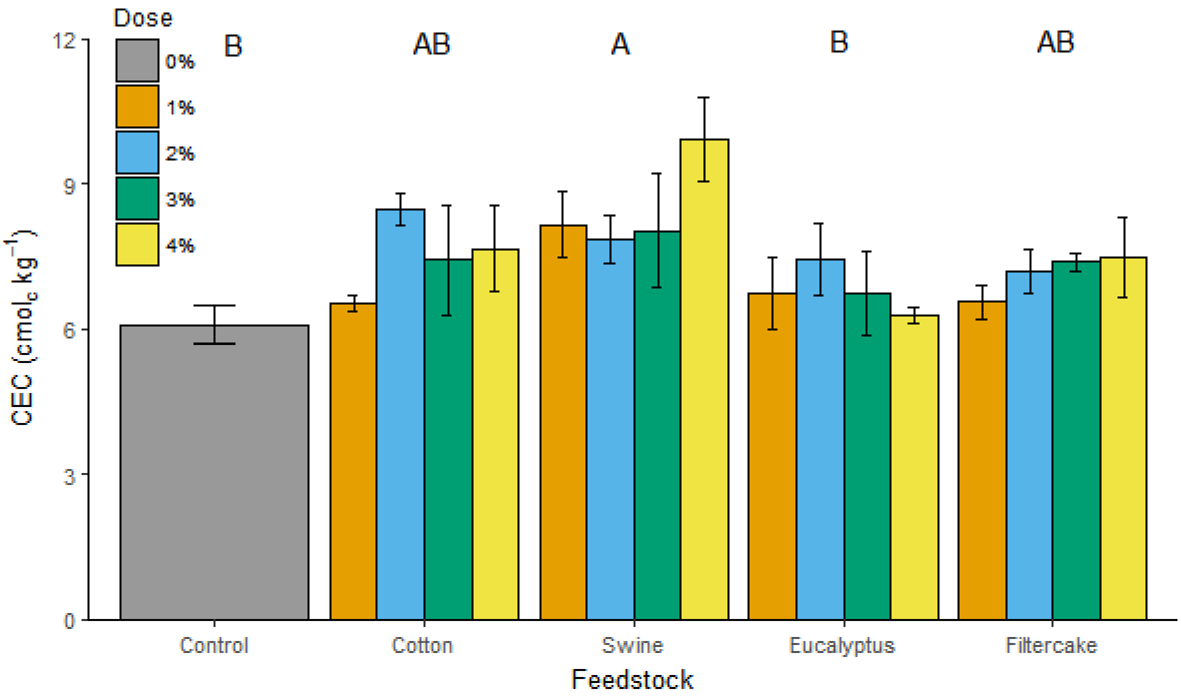

Figure 3.2. Mean cation exchange capacity (CEC; cmol$_c$ kg^{-1}) of soils with different biochar feedstocks and doses after 6 weeks (*n* = 4). Capital letters indicate significant differences between the feedstocks (Tukey test; *P*<0.05).

Mean soil EC was highest in soils with cotton and swine manure biochars compared to eucalyptus and filtercake biochars and the control. EC between the doses were different only for cotton and swine manure biochars, where the EC was generally higher in the high doses compared to the lower doses (Figure 3.3). EC was significantly (*P* < 0.001) and negatively correlated with both dry aboveground biomass (R = -0.81) and dry belowground biomass (R = -0.81), while positively correlated with soil pH (R = 0.81, *P* < 0.001) (Appendix Figure E.2).

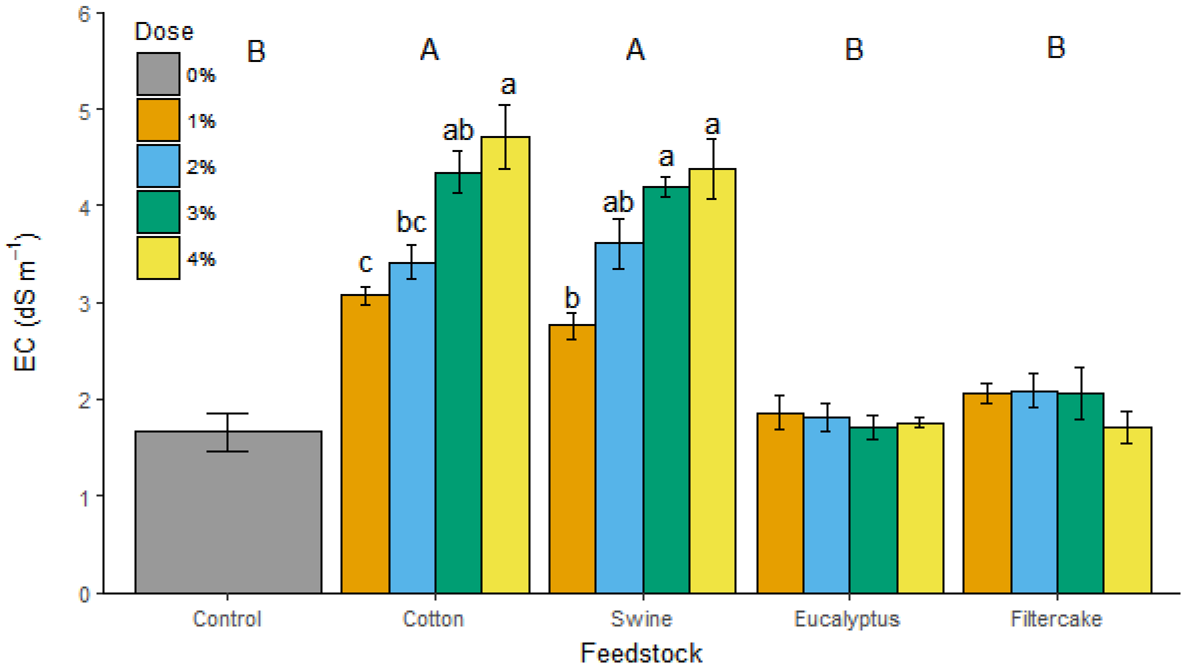

Figure 3.3. Mean electrical conductivity (EC, dS m^{-1}) measured 3 times a week for 5 weeks (*n* = 4). Capital letters represent significant differences between the feedstocks while lowercase letters represent significant differences between the doses (Tukey test; *P* < 0.05).

Above and belowground dry biomass had negative correlations with total C and several soil nutrients (Table 3.3, Appendix Figures E.3, E.4, E.5). Dose and feedstock were both significant factors for soil total C levels (Figure 3.4A). Cotton (1.5±0.06 %C) and eucalyptus (1.4±0.09 %C) biochars increased soil total C more than filtercake biochar (1.2±0.04 %C), but not more than swine manure biochar (1.3±0.1 %C). All biochars significantly ($P < 0.05$) increased soil total C more than the control (0.8±0.03 %C), and doses showed a trend of increasing C with increasing dose (Figure 3.4A). Biochar feedstock and dose effects varied for soil macronutrients, including total N. For total N, soils with swine manure biochar had the greatest N levels (0.06±0.01%N), significantly ($P < 0.05$) greater than the control (0.02±0.00 %N) and eucalyptus biochar (0.02±0.00 %N), but not different from cotton (0.04±0.00 %N) and filtercake (0.04±0.01 %N) biochars. Only swine manure biochar showed significant differences between the doses for soil N, with the smallest dose (1%) significantly ($P < 0.05$) lower than the largest dose (4%) (Figure 3.4B).

Table 3.3. Pearson correlation coefficients (R) for above and belowground biomass with total C and soil nutrients. * = *P* < 0.05, ** = *P* < 0.01, ns = not significant

	C (%)	**N (%)**	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Ca (cmol$_c$ kg^{-1})**	**Mg(cmol$_c$ kg^{-1})**	**S (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Mn(mg kg^{-1})**	**B (mg kg^{-1})**
Aboveground dry biomass	-0.46**	-0.25*	-0.03^{ns}	-0.71**	0.34**	-0.21^{ns}	-0.73**	-0.41**	-0.44**	0.48**	0.17^{ns}	-0.30*
Belowground dry biomass	-0.49**	-0.28*	-0.06^{ns}	-0.72**	0.34**	-0.17^{ns}	-0.70**	-0.37**	-0.40**	0.38**	0.18^{ns}	-0.29*

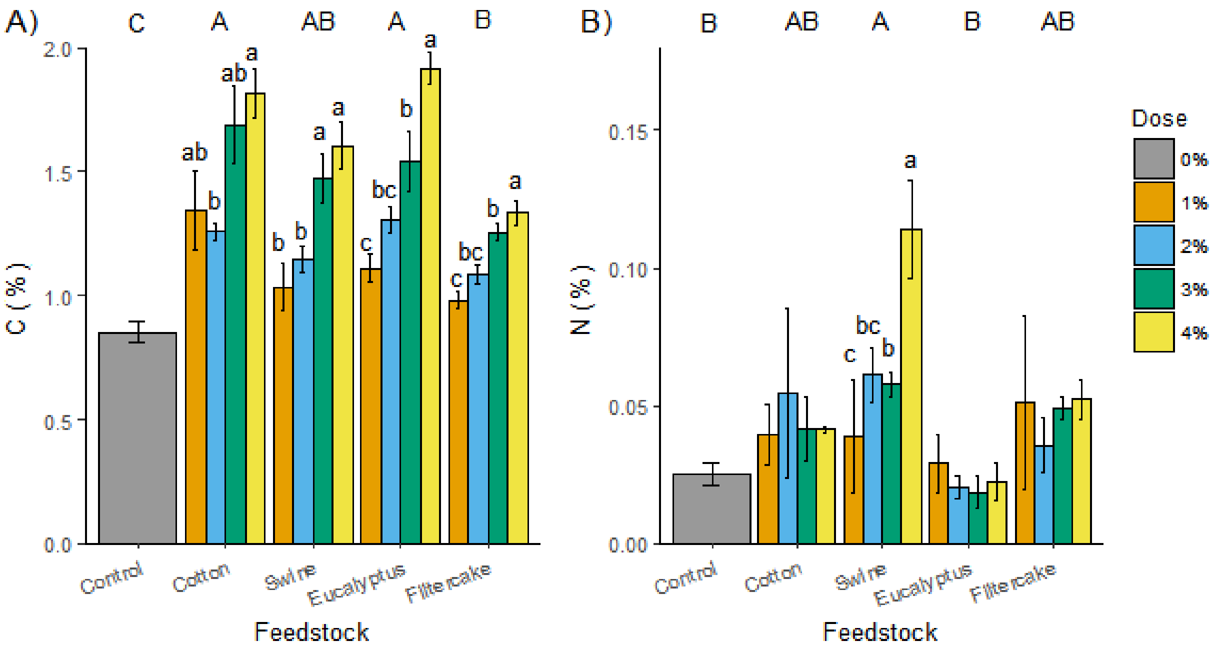

Figure 3.4. A) Mean soil total carbon (C; %) and B) mean soil total nitrogen (N; %) in soils with different biochar feedstocks and doses (*n* = 4). Capital letters indicate significant differences between the feedstocks and lowercase letters indicate significant differences between doses for each feedstock (Tukey test; *P*<0.05).

Mean soil C/N ratios reflected the high total C levels in soils with eucalyptus and cotton biochars, with mean C/N ratios in eucalyptus biochar treatments (92.4±15.3) not different from cotton biochar treatments (45.7±5.7), but significantly greater than swine manure (27.1±5.1) and filtercake (35.0±6.7) biochar treatments and the control (36.6±6.8). For phosphorus (P) levels, there were no significant differences between the feedstocks nor the doses (Figure 3.5A). Potassium (K) levels in soils with cotton (1182±153 mg K kg^{-1}) and swine manure (785±127 mg K kg^{-1}) biochars were significantly higher than in soils with eucalyptus (251±35 mg K kg^{-1}) and filtercake (227±24 mg K kg^{-1}) biochars, and higher compared to the control (157±11 mg K kg^{-1}) (Figure 3.5B). Calcium (Ca) and magnesium (Mg) showed no differences between the doses, but had significant differences ($P < 0.05$) between the feedstocks (Figure 3.5C and D). Soils amended with filtercake biochar (3.2±0.1 cmol$_c$ kg^{-1}) had significantly more Ca than cotton (1.7±0.1 cmol$_c$ Ca kg^{-1}) and eucalyptus (2.6±0.1 cmol$_c$ Ca kg^{-1}) biochars, but not more than swine manure biochar (2.8±0.1 cmol$_c$ Ca kg^{-1}); filtercake biochar was the only feedstock to significantly ($P < 0.05$) increase Ca levels in the soil compared to the control (2.5±0.3 cmol$_c$ Ca kg^{-1}). Like Ca, Mg levels also did not differ between doses and only soils with swine manure biochar (1.8±0.4 cmol$_c$ Mg kg^{-1}) had significantly ($P < 0.05$) higher Mg levels than the other treatments which did not differ from each other. For sulfur (S), soils with cotton and swine manure biochar had significantly ($P < 0.05$) greater levels than eucalyptus and filtercake biochars and were significantly greater than the control. Only swine manure biochar had differences between the doses, where soil S levels increased with increasing dose (Figure 3.6A).

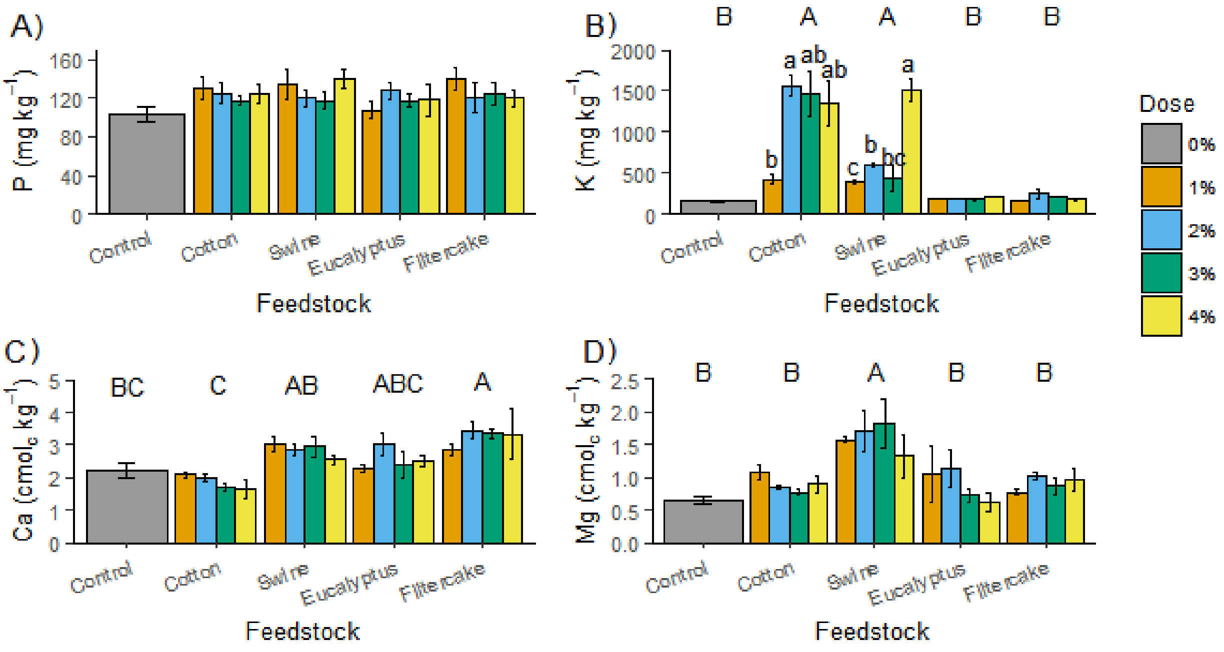

Figure 3.5. Mean macronutrients: A) phosphorus (P; mg kg^{-1}), B) potassium (K; mg kg^{-1}), C) calcium (Ca; cmol$_c$ kg^{-1}), and D) magnesium (Mg; cmol$_c$ kg^{-1}) in soils with different biochar feedstocks and doses after 6 weeks (*n* = 4). Capital letters indicate significant differences between the feedstocks and lowercase letters

indicate significant differences between doses for each feedstock (Tukey test, P < 0.05; Games-Howell test, P < 0.05 for K and Mg); where absent, differences were not significant.

Biochar feedstock type had a significant (*P* < 0.001) effect on all micronutrients except boron (B), while differences in doses were mostly observed for swine manure biochar on certain micronutrients (Figure 3.6B-F). For both zinc (Zn) and copper (Cu), soils with swine manure biochar had significantly (*P* < 0.05) higher levels than the other biochars which did not differ from each other nor from the control. The doses exhibited the same trend in swine manure biochar as seen for S (Figure 3.6B and C). For iron (Fe), soils with cotton (54±3.1 mg Fe kg^{-1}), swine manure (53±3.7 mg Fe kg^{-1}), and eucalyptus (54±3.4 mg Fe kg^{-1}) had significantly (*P* < 0.05) lower Fe levels than filtercake biochar (117±6.5 mg Fe kg^{-1}), the latter also being higher than the control (47±4.4 mg Fe kg^{-1}) (Figure 3.6D). For manganese (Mn), soils with swine manure and filtercake biochars had significantly (*P* < 0.05) higher Mn levels than cotton and eucalyptus biochars and the control. Both swine manure and filtercake biochars had significant (*P* < 0.05) differences between the lowest and the highest doses (Figure 3.6E). Lastly, soil boron (B) levels did not significantly differ between the biochar feedstocks, doses, or the control (Figure 3.6F).

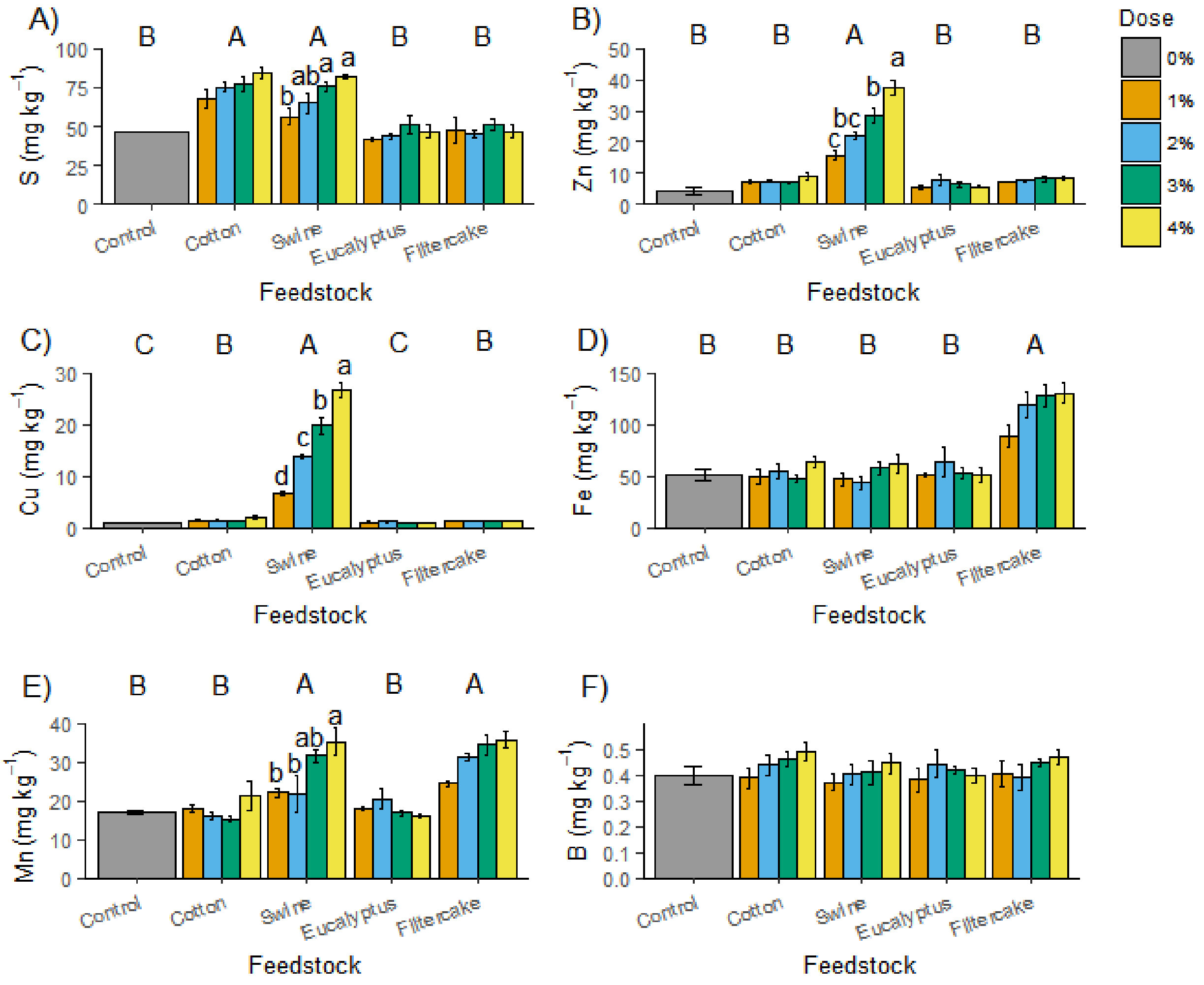

Figure 3.6. Mean concentration of A) sulfur (S; mg kg^{-1}) and micronutrients: B) zinc (Zn; mg kg^{-1}), C) copper (Cu; mg$_c$ kg^{-1}), D) iron (Fe; mg kg^{-1}), E) manganese (Mn; mg kg^{-1}), and F) boron (B; mg kg^{-1}) in soils with different biochar feedstocks and doses after 6 weeks (*n* = 4). Capital letters indicate significant differences between the feedstocks and lowercase letters indicate significant differences between doses for each feedstock (Tukey test, *P* <0.05, for A and F; Games-Howell test, *P* < 0.05, for B-E) where absent, differences were not significant.

3.3.2 Physiological properties

Among measured physiological variables (P_n, g_s, R_d, E, WUE_s, and WUE_t), the repeated measures ANOVA showed a significant overall treatment effect only for R_d ($P < 0.05$). The time effect was also significant ($P < 0.05$) for most variables except for g_s and R_d. Differences between the time*treatment interaction (i.e., the treatment effects were different on day 20 compared to on day 42) were significant ($P < 0.05$) for P_n, g_s, and WUE_t.

Treatment differences were significant for several final plant physiological and physical characteristics (Table 3.4). Maize plants in soils with cotton and some swine manure biochars were consistently smaller and with less biomass than the other biochars and the control. Cotton biochar had the lowest WUE_{Prod} and filtercake biochar the highest, while the Σwater consumed, NUE_{Prod}, and $PNUE_{plant}$ were also lowest for cotton biochar.

P_{plant} was significantly correlated with total fresh leaf biomass (R=0.54, $P < 0.05$) and with SLA (R=0.58, $P < 0.01$), as well as with three soil properties: soil K (R=0.73, $P < 0.01$), S (R=0.57, $P < 0.01$), and soil pH (R=0.54, $P < 0.05$) (Appendix Figure E.6). Biochar treatments with low fresh leaf biomass (e.g. cotton and swine manure biochars) had lower P_{plant} rates than other treatments. SLA, however, did not differ significantly between treatments, nor did leaf C:N ratio. There was a positive correlation between P_{plant} and the ∑water consumed (R=0.75, $P < 0.001$) and NUE_{prod} (R=0.82, $P < 0.001$), as well as between WUE_{Prod} and NUE_{Prod} (R=0.75, $P < 0.001$).

Table 3.4. Physiological and physical properties of maize plants (1% biochar dose) at the end of the experiment (day 42). Values are means± 1 SE (*n* = 4). Different lowercase letters represent significant differences between the treatments (Tukey test, $P < 0.05$; Games-Howell test for stem diameter, plant height, and total dry leaf biomass, $P < 0.05$).

Plant physiological properties

Treatment	P_{plant} (μmol(CO_2)·m^{-2}·s^{-1}·mm^{-2})	R_{plant} (μmol·m^{-2}·s^{-1}·mm^{-2})	E_{plant} (mmol(H_2O) ·m^{-2}·s^{-1}·mm^{-2})	g_{plant} (mol(H_2O)·m^{-2}·s^{-1}·mm^{-2})	∑water consumed (L)	WUE_{Prod} (g L^{-1})	NUE_{Prod} (g mg^{-1} leaf N)	$PNUE_{plant}$ (μmol(CO_2)·g N^{-1} ·s^{-1})
Control	15,146.2±1410.6^{a}	-487.8±65.8^{ab}	1,924.7±265.1^{a}	125.3±19.0^{a}	3.5±0.2^{a}	4.4±0.2^{abc}	5.4±0.6^{a}	5022.2±388.9^{ab}
Cotton1%	6,940.0±331.5^{c}	-173.3±12.5^{c}	855.4±75.1^{c}	52.6±3.7^{c}	2.7±0.1^{b}	3.4±0.2^{c}	3.1±0.2^{b}	2259.9±126.7^{c}
Swine1%	11,556.5±799.3^{b}	-352.2±34.4^{b}	1,350.7±142.1^{bc}	83.1±8.5^{bc}	3.0±0.1^{ab}	3.9±0.6^{bc}	4.5±0.3^{ab}	3926.9±221.7^{b}
Eucalyptus1%	14,540.0±1118.6^{ab}	-466.8±4.04^{ab}	1,733.5±154.2^{ab}	110.8±9.5^{ab}	3.4±0.1^{a}	4.7±0.4^{ab}	5.8±0.6^{a}	5137.6±458.6^{a}
Filtercake1%	16,332.8±1262.3^{a}	-549.8±45.0^{a}	2.083.3±226.3^{a}	128.0±13.0^{a}	3.4±0.2^{a}	5.3±0.3^{a}	6.5±0.5^{a}	5655.1±545.6^{a}

Plant physical properties

Treatment	Total LA (mm^2)	Stem diameter (mm)	Number of leaves	Plant height (cm)	Total fresh leaf biomass (g)	Total dry leaf biomass (g)
Control	3,096.6±332.5^{ab}	12.0±0.6^{a}	12.3±0.7^{ab}	178.3±7.8^{ab}	68.4±6.0^{a}	9.4±1.1^{a}
Cotton1%	1,528.8±32.9^{c}	9.5±0.3^{b}	10.5±0.5^{b}	136.3±2.9^{c}	38.2±0.5^{b}	5.2±0.1^{b}
Swine1%	2,310.0±108.9^{bc}	12.3±0.3^{a}	12.0±0^{ab}	151.0±3.2^{bc}	53.5±2.0^{ab}	7.4±0.2^{ab}
Eucalyptus1%	2,977.5±232.0^{ab}	11.5±0.3^{a}	12.8±0.3^{a}	172.0±3.4^{a}	65.2±3.7^{a}	9.3±0.6^{a}
Filtercake1%	3,288.6±232.0^{a}	12.5±0.7^{a}	13.0±0.4^{a}	175.8±3.1^{a}	65.3±5.9^{a}	9.2±0.9^{a}

Dark respiration rate at the leaf scale varied significantly ($P < 0.05$) between treatments, but not over time, except for the cotton treatment (means compared by *t*-test, $P < 0.001$). Other biochars showed lower respiration at the end of the experiment compared to mid-experiment as well (Figure 3.7). Cotton biochar reduced R_d compared to the control and eucalyptus and filtercake biochars. R_d was also significantly correlated with leaf C (R =0.47, $P < 0.05$) and with total fresh leaf biomass (R=0.69, $P < 0.001$). At the plant scale, plants in soils with cotton biochar had the lowest R_{plant}, g_{plant}, and E_{plant} rates, while plants in soils with filtercake biochar had the highest, although these were not significantly different from the other two biochars or the control (Table 3.4). R_{plant}, g_{plant}, and E_{plant} rates were significantly correlated with Σwater consumed (R_{plant}: R =0.79, $P < 0.001$; g_{plant}: R =0.63, $P < 0.001$; E_{plant}: R =0.59, $P < 0.001$).

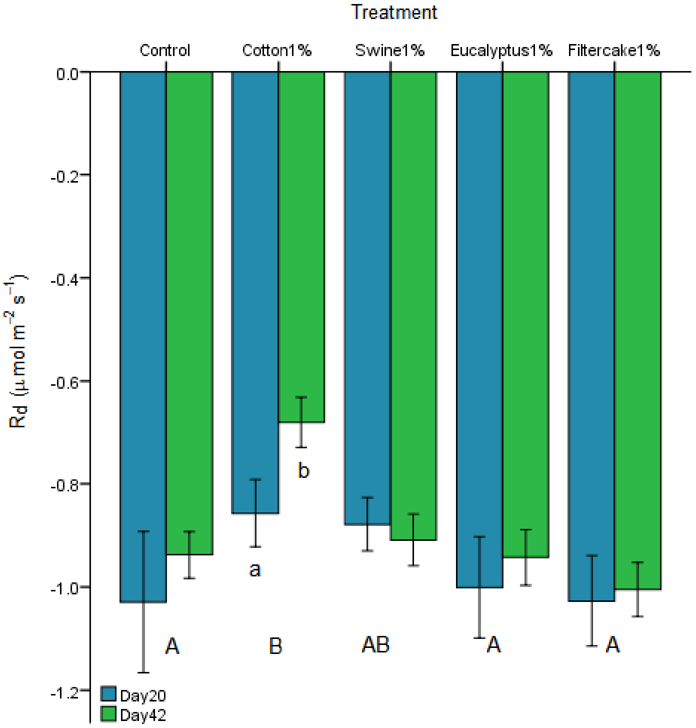

Figure 3.7. Mean dark respiration rate (R_d, µmol m^{-2} s^{-1}) for maize plants in soils with different biochar and control (no biochar) treatments measured halfway (day 20) and at the end (day 42) of the experiment. Capital letters represent significant differences (Tukey test; $P < 0.05$) between treatments. Lowercase letters represent significant differences between day 20 and 42 for a treatment (t-test, $P < 0.001$).

3.3.3 Principal component analysis

The PCA of soil and plant properties of biochar treatments applied at a 1% rate and the control found two groupings: R_{plant}, S, EC, K, pH, Zn, and Cu (Group 1) and $PNUE_{plant}$, P_{plant}, E_{plant}, g_{plant}, total LA, dry aboveground biomass, plant height, and Ca/Mg ratio (Group 2). Cotton and swine manure biochars clustered by Group 1, while eucalyptus and filtercake biochars and the control clustered strongly by Group 2 (Figure 3.8).

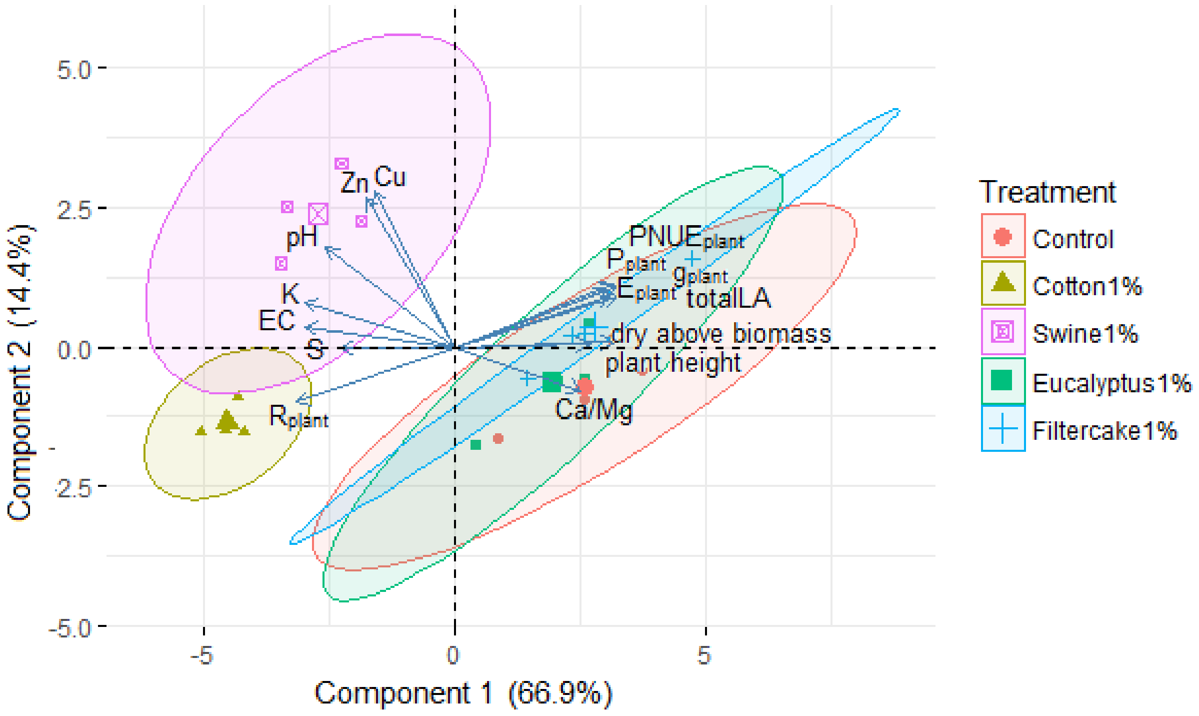

Figure 3.8. Principal component analysis (PCA) of soil and plant properties of 400°C biochar treatments at 1% application rate and the control (*n* = 4).

3.4 Discussion

As all soils were fertilized and adequately watered, including the control, this experiment highlighted which biochars and application rates could have a negative effect on plant growth when combined with standard fertilizing practices in a Cerrado Arenosol. In addition, receiving adequate watering suggests which biochars could help retain soil water remaining from the wet season moving into the dry season, maintaining maize growth. Nevertheless, some biochars increased soil nutrient contents and led to similar or even higher (e.g. filtercake biochar at 4%) mean maize biomass compared to the control.

3.4.1 Biochar effects on soil pH, CEC, and EC

Soil acidity is one of the main limiting factors for crop production in tropical soils such as those of the Cerrado, which have an average pH of 5. Liming is often practiced in Cerrado soils to raise pH levels (Fageria, 2001) by increasing Ca^{2+} and Mg^{2+} content, thus reducing soil acidity (Frazão et al., 2008) and improving CEC (Silber et al., 2010). Prior studies have shown a similar liming effect of biochars in nutrient-poor soils due to the high pH of the biochars themselves which tend to be alkaline (Biederman and Harpole, 2013; Lehmann et al., 2003; Van Zwieten et al., 2010). Initial soil pH in our study was 5.8 (Table 3.1), suitable for plant growth, but by the end of the study decreased to 4.9 (Table 3.2). In contrast, in soils with biochar a liming effect was evident, where the high pH of three of the four biochars (pH for cotton biochar = 10, swine manure biochar = 9.2, and filtercake biochar = 9.0) (Table 3.1), significantly increased the soil pH in biochar treatments compared to the control soil after 6 weeks (Table 3.2). The pH levels of biochars used in this study were comparable to those used by Rajkovich et al. (2012), where corn stover and animal manure (dairy and poultry) biochars had higher pH levels than wood biochars (oak and pine). The beneficial liming property of biochar can sometimes be deleterious, however, if biochar is over applied (Chan and Xu, 2009). Maize biomass decreased with increasing application dose in our study for cotton and swine manure biochars, and was negatively correlated with increasing soil pH (Appendix Figure E.1A), suggesting the resulting higher soil pH levels may have affected nutrient availability for plant uptake.

Biochar is known to increase soil CEC, improving nutrient retention (Biederman and Harpole, 2013). As our control soil was not experiencing nutrient deficiency, CEC levels under most biochar treatments were not significantly higher than the control (except for swine manure biochar treatments) and the correlation between CEC levels and biomass was low (Appendix Figure E.1B). Xu et al. (2015) similarly observed that peanut shell biochar increased availability of certain nutrients (e.g. P, K) in two types of soils (red ferrosol and redoxi-hydrosol), but did not affect soil CEC. However, CEC has been shown to increase after a few months following biochar addition (Cheng et al., 2008), thus the short experimental timeframe (5 months in Xu et al. (2015) and 2 months in our study), likely did not provide enough time for oxidation of biochar surfaces.

EC can be used to indicate salinity and determine crop yield potential. High salinity can reduce plants' ability to uptake water and disrupt their nutritional balance (Corwin and Lesch, 2005). The high EC in soils with cotton and swine manure biochars, along with their low plant biomass and the significant negative correlation, suggest that these biochars led to excessive soil salinity which, combined with high pH and other factors, may also have impacted plant growth (Appendix Figure E.2). Using biochar as a potting soil, Blok et al. (2017) observed that biochars made from nutrient-rich feedstocks were not suitable due to their high salt content and pH. The authors suggested that feedstock salinity and alkalinity can be tested before biochar production, although a biochar's high EC can still be lowered by prior washing with water or by combining with a lower EC biochar. In addition, lower biochar applications in the field would reduce the effects of salinity and pH (Blok et al., 2017).

3.4.2 Biochar effects on soil macronutrients

Cotton and swine manure biochars had the lowest dry above- and belowground biomass, yet had higher or similar concentrations of soil nutrients than eucalyptus and filtercake biochar in certain cases, again suggesting that nutrient availability was affected in these soils or that excessive nutrient levels caused plant toxicity. Moreover, the poor correlation between high soil total C and low maize biomass particularly observed for cotton biochars at high application rates (Appendix Figure E.3A) suggests that cotton biochar may alter soil physical properties that affect nutrient availability, such as water retention (see **Section 2.4.2**). Soil total N also had a poor correlation with decreasing maize biomass, mostly due to swine manure biochar's contribution to high total N, but low plant biomass (Appendix Figure E.3B). Its weak relationship with plant biomass and swine manure biochar's high contribution to soil total N levels with increasing application rates, as well as its low soil C/N ratio, suggest that soil N deficiency did not lead to the low plant biomass in soils with cotton and swine manure biochars. In addition, all soils received inorganic NPK fertilizer. Although available N was not measured in the present study, it has been reported in the literature that biochars provide negligible amounts of available N in the form of nitrate (Ippolito et al., 2015). Thus increased soil total N in swine manure biochar treatments may have been due to increased N immobilization in microbial biomass (Steiner et al., 2008).

In contrast to soil N, soil available P levels in our study did not differ between the biochars nor did the biochars increase or decrease P levels in the soil compared to the control. Although higher pH can increase P availability by reducing P absorption and allowing P desorption from Al and Fe oxides (Xu et al., 2015), this was not observed in the present study and neither was P significantly correlated with maize biomass (Table 3.3). Nevertheless, the effect of biochar on P is not yet well understood, as studies have either reported increased (Novak et al., 2009) or decreased (Nelson et al., 2011) P adsorption. In a meta-analysis of over 300 experiments observing the effect of biochar on plant productivity and nutrient cycling, Biederman and Harpole (2013) found that biochar applications significantly ($P < 0.01$) increased soil P and K compared to control soil without biochar. Although P levels in the soil-biochar mixtures did not differ in our study, cotton and swine manure biochars increased soil K more than eucalyptus and filtercake biochars, and more than the control, but increasing soil K levels were correlated with decreasing maize biomass (Table 3.3), particularly for cotton biochar (Appendix Figure E.4). High soil K levels may have affected plant growth, altering plant photosynthesis as discussed below in **Section 3.4.4**.

Like soil K, soils with both cotton and swine manure biochar also had high S levels, which had a strong negative correlation with maize biomass (Table 3.3, Appendix Figure E.4). The decrease in maize biomass may have been caused by S toxicity. As in our study, Chandra and Pandey (2016) observed that S toxicity (as well as S deficiency) in soybean plants caused reduced biomass with increasing S doses. For maize production in Brazil, soils with less than 10 mg S kg^{-1} receive S fertilizers (typically as ammonium sulfate) (Coelho et al., 2011). Maize plants in our study grew well at levels around 45 mg S kg^{-1}, but were visibly smaller in soils with cotton and swine manure biochar (Appendix Figure F.1 and F.2), particularly in higher biochar doses where S levels almost doubled that of the control. Biochar has been known to decrease plant yield in certain instances, specifically in relation to high S levels and salinity, Al/Mn toxicity, and reduced nutrient availability (Jeffery et al., 2015a). In contrast, Ca levels had a low, but positive correlation with maize biomass (Table 3.3) and were highest in soils with swine manure and filtercake biochars. However, higher soil Ca levels appear to have contributed to greater maize biomass mostly for plants in soils with filtercake biochar (Appendix Figure E.4). Swine manure biochar also contributed to the highest soil Mg compared to the other biochars, but as Mg was not significantly correlated with maize biomass, it likely did not cause toxicity affecting plant growth.

As mentioned, increasing applications rates resulted in a negative trend of decreasing maize dry above- and belowground biomass for cotton and swine manure biochars, but no trend for eucalyptus and filtercake biochars. Rajkovich et al. (2012) likewise reported that above a 2% (w/w) application rate, their different biochars had negative, positive, or no effect on corn growth. For filtercake and eucalyptus biochars, application rates ranging from 1 to 4% (w/w) did not have varying effects on maize biomass. This may be related to pH levels in soils with filtercake and eucalyptus biochars which remained relatively consistent with increasing dose, whereas pH in soils with cotton and swine manure biochar increased significantly with increasing dose. Thus, lower biochar application rates (e.g. <1% w/w) for some biochars may help improve plant biomass, and could potentially be blended with other biochars to obtain desired soil conditions for crop productivity.

3.4.3 Biochar effects on soil micronutrients

Micronutrient availability is particularly affected by pH levels (less micronutrient availability with increasing pH), while macronutrient (N, K, S) availability is affected to a lesser extent (Jensen, 2010). Although increased soil pH caused by some biochars' alkalinity can lead to soil N losses through ammonia (NH_3) volatilization (Chen et al., 2013), the biochar treatments in our study did not appear to experience N losses (Figure 3.4B). In addition, when mixed with raw wastes such as sewage sludge, biochar was observed to significantly reduce N losses (Hua et al., 2009), perhaps due to adsorption of NH_3 (Taghizadeh-Toosi et al., 2011). Excessive micronutrient levels, however, can cause toxicity and negative plant growth. The heavy metals, Cu and Zn, are essential micronutrients for plant growth, but if they are present at higher levels than the plant requires, they can cause toxicity (Nagajyoti et al., 2010). For Cerrado soils, average Cu levels are between 0.8 and 2.4 mg kg^{-1} and between 1 and 3 mg kg^{-1} for Zn (Coelho et al., 2011). Initial Cu levels in our study were within the average range (0.97±0.3 mg kg^{-1}), but increased significantly under swine manure biochar (Figure 3.6C). Excessive Cu levels can cause chlorosis (leaf yellowing) and decrease plant biomass (Adrees et al., 2015), which was evident for maize plants grown in soils receiving cotton or swine manure biochars (Table 3.3; Appendix Figure F.5 and F.6). However, Cu levels in soils with cotton biochar were not excessively high, suggesting Cu toxicity was not the cause of the decrease in maize biomass under that biochar. Surprisingly, although Cu solubility is highly dependent on pH, decreasing with increasing alkalinity (Adrees et al., 2015), soil Cu levels under swine manure biochar increased with rising dose application and pH (Table 3.1; Figure 3.6C). Like Cu (and other micronutrients), Zn is also increasingly soluble in more acidic soils (Brady and Weil, 2002). Zn levels in our initial soil were at the high end (2.8±0.9 mg kg^{-1}) and were higher by the end of the experiment, again particularly under swine manure (Figure 3.6B) despite increasing pH. According to Fageria (2000), however, maize has a high tolerance to Zn toxicity compared to other crops such as rice, soybean, and wheat, withstanding a soil Zn toxic level up to 60 mg kg^{-1}. Zn levels in soils with swine manure biochar were high, but did not reach toxic levels. Signs of Zn toxicity, like Cu, also include low growth and development (Nagajyoti et al., 2010), but both micronutrients' relationship with maize biomass had low R^{2}s in our study (Table 3.3, Appendix Figure E.5). Increased Zn solubility could be beneficial in Cerrado soils since Zn is the main limiting micronutrient for maize production in Brazil, particularly in the Cerrado region (Coelho et al., 2011). Adding a low dose of swine manure biochar to the soil therefore could help raise Zn bioavailability. In addition, combining swine manure with filtercake or eucalyptus residue could perhaps produce a biochar that contributes Cu and Zn while increasing total N and C, soil organic matter, and plant biomass. Mixed biochars made from combinations of these feedstocks remain to be tested.

Another heavy metal considered an important micronutrient in trace amounts is iron; it is essential in metabolic processes (Nagajyoti et al., 2010; Rout and Sahoo, 2015). Excess uptake, however, can cause toxicity in plants (Rout and Sahoo, 2015). Average Fe levels in Cerrado soils are 5 to 12 mg kg^{-1}, with levels above 12 mg kg^{-1} considered high (Coelho et al., 2011). Our initial soil had high Fe levels (79.0±4.5 mg kg^{-1}), which decreased by the end of the experiment, except in soils with filtercake biochar (Figure 3.6D). Soil Fe levels were only high in soils with filtercake biochar, including compared to the control. Despite the high levels, Fe did not seem to have a detrimental effect on plant growth (Appendix Figure E.5) as maize plants in soils with filtercake biochar still had high above- and belowground biomass comparable to the control, and no signs of toxicity or deficiency compared to the other biochar treatments (Appendix Figure F.3). Fe is not readily available in neutral to alkaline soils, as it is in insoluble oxidized forms, e.g. Fe^{+3} (Rout and Sahoo, 2015). Although soils under filtercake biochar treatments remained slightly acidic (Table 3.2), the high soil Fe was likely unavailable for plant uptake, yet it appeared to be available in sufficient amounts to prevent Fe deficiency.

Both filtercake and swine manure biochar had significantly high levels of Mn compared to the control and the other biochars. Mn contributes to photosynthesis, N assimilation and metabolism (Brady and Weil, 2002). In Cerrado soils with pH around 6, average Mn levels are 5 to 15 mg kg^{-1} (Coelho et al., 2011). Mn levels in soils with the higher doses of swine manure and filtercake biochar were much higher than 15 mg kg^{-1}, but plants under the filtercake biochar treatment did not appear affected (Appendix Figure F.3) and there was no significant correlation between Mn and maize biomass (Table 3.3). Signs of Mn toxicity are leaf browning or spotting, chlorosis, and “crinkle-leaf” (Nagajyoti et al., 2010). Plants under eucalyptus biochar treatments also did not show these symptoms (Appendix Figure F.4). Some of these signs were visible in maize plants with swine manure biochar (Appendix Figure F.5), and crinkle-leaf and browning leaf tips were also visible in plants with cotton biochar (Appendix Figure F.6). Mn levels in soils with cotton biochar were not significantly greater than the control, thus the symptoms were probably not due to Mn toxicity. Soil B levels likely also had little impact on maize biomass, as reflected by its low correlation (Table 3.3, Appendix Figure E.5). However, in well-aerated, calcareous (thus alkaline) soils, there can sometimes be a deficiency in available Fe, Zn, and Mn despite there being adequate levels of these micronutrients (Brady and Weil, 2002). To summarize, the poor growth and development in maize plants under cotton and swine manure biochar treatments at high doses may have been caused by either micronutrient deficiencies due to high pH, toxicity from high S levels, high K levels, or excessive salinity. More likely, a combination of these factors affected maize plants as suggested by the PCA of biochar treatments at the 1% application rate where pH, EC, S, and K were negatively correlated and pH, Zn and Cu were positively correlated; both cotton and swine manure biochars clustered closely to this group (Group 1) (Figure 3.8). The availability of these nutrients for plant absorption would also be related to water availability, which is discussed below.

3.4.4 Biochar effects on physiological properties

Since biochar can alter nutrient availability in the soil, it can likely also affect photosynthesis and plant production (Xu et al., 2015). Kammann et al. (2011) observed that biochar applications reduced transpiration rates and maximum apparent photosynthesis in sandy soils with both 20%

and 60% water-holding capacity compared to controls with no biochar. In our study, biochar treatments had no effect on P_n or E, nor on g_s measured at the leaf scale. When P_n was considered with total LA (P_{plant}), however, cotton biochar had the lowest P_{plant}, although its total LA did not differ significantly from plants in soils with swine manure biochar (Table 3.4). P_{plant} was significantly correlated with soil K and S, where biochars with high soil K and S (cotton and swine manure) had low P_{plant} compared to the other two biochars. Sulfur is essential for plant growth and metabolism, and sulfur deficiency can lead to reduced photosynthesis rates (Resurreccion et al., 2001). Potassium affects water relations in plants through its role in stomatal opening, and K deficiency can cause lower leaf P_n and g_s (Lu et al., 2016), reducing WUE (Kammann and Graber, 2015). In theory, if a biochar contributes to soil K, it could alleviate drought stress by improving g_s and plant osmotic potential. Biochar-derived K is highly soluble, however, and can be easily leached (Kammann and Graber, 2015). In our study, since cotton and swine manure biochar treatments had the highest soil K and S levels, but the lowest P_{plant} rates, the soil K and S were either not plant available and causing deficiency, or were too high causing toxicity and thus also impairing P_{plant}.

Another explanation for the low P_{plant} and low biomass in general of plants with cotton and swine manure biochars may be related to their lower Σwater consumed and in turn, WUE_{Prod}. The fact that soils containing these biochars remained visibly saturated for longer periods between waterings than the other two biochars and the control, therefore requiring less water additions, suggests that they improved soil water retention. However, based on the low biomass, LA and WUE_{Prod}, the water retention may have been too high (particularly in soils with cotton biochar which had a high C/N ratio), causing the plants to suffer. Biochar is considered a porous material and its porosity is determined both by the feedstock (which contributes mostly to macropores) and to the temperature of pyrolysis (which contributes to nanopores) (Brown et al., 2015; Uzoma et al., 2011b). Residual macropores (1 to 100µm) are formed from plant cellular structure and are believed to contribute to biochar pore volume, while pyrogenic nanopores (less than 50 nm) contribute mostly to biochar surface area (Gray et al., 2014). When added to the soil, biochar has the potential to increase pores in the 30 to 0.3 nm diameter range (Verheijen et al., 2009). Yet, for plant available water content (AWC, water content at field capacity minus water content at the permanent wilting point) (Rajkovich et al., 2012), the pore size range is 0.2 to 30µm diameter; most plants are not able to take up water from pores smaller than 0.2 µm (Hardie et al., 2014). Thus, although biochar can improve the soil porosity, depending on the biochar, the effective plant AWC may not vary in response to biochar additions (Verheijen et al., 2009). Further, depending on the biochar, water can bind to its particle surface, increasing water storage, but keeping the water immobile and unavailable for plants or solute transport (Masiello et al., 2015).

Studies have shown that biochar improves WUE (Kammann et al., 2011; Laghari et al., 2015; Uzoma et al., 2011a). In our study, however, none of the biochars significantly improved WUE_s, WUE_t, or WUE_{Prod} compared to the control. In a corn growth experiment with various biochars made of different feedstocks and temperatures of pyrolysis, Rajkovich et al. (2012) observed that AWC was neither correlated with plant growth nor that AWC in biochar-soil mixtures increased compared to the control. The authors suggested this was due to the fact that soil water was kept at optimum levels for all treatments to prevent plant stress, as was the case in our study. AWC in their case, however, did vary between biochars. In our study, AWC did not vary significantly between the treatments, including the control (Appendix Figure G.1), although soils with cotton and swine manure biochar had slightly higher mean AWC values. In most laboratory or greenhouse studies, biochar amendments ≥ 4% w/w are needed in order to see a significant increase in AWC (Kammann and Graber, 2015). Though not significant at the 1% rate, slightly higher mean AWC values in these biochar treatments may have caused short-term anoxic conditions that impacted plant growth, especially at higher doses. Observationally, water in pots with cotton and swine manure biochars at higher doses required more time to percolate than in pots with the other biochars. In addition, plants in soils with cotton and swine manure biochars had lower mean dry belowground biomass than the other biochars and the control. Wet or oxygen-poor soils can impede root growth, reducing plant water supply (Kammann and Graber, 2015). As nutrient concentrations were similar or even higher (e.g. K and S) in soils with cotton and swine manure biochars compared to soils with eucalyptus and filtercake biochars, it is possible that soil water retention also played a role in plant productivity, along with pH and nutrient availability.

Like P_{plant}, R_{plant}, g_{plant}, and E_{plant} were lowest in soils with cotton biochar and highest with filtercake biochar (Table 3.4). These properties are also affected by soil water level and leaf N content (Kammann et al., 2011). In our study, R_{plant}, g_{plant}, and E_{plant} were not correlated with leaf N, but they were significantly correlated with Σwater consumed, especially R_{plant}. All three properties increased with increasing Σwater consumed, in agreement with Kammann et al. (2011) who observed reduced stomatal conductance, transpiration, and R_{leaf} in low soil water availability (20% WHC) where Σwater consumed was lowest.

Nitrogen is often a limiting factor in agricultural soils and is essential for plants, including maize (Zheng et al., 2013). Reducing N losses is therefore important to agricultural producers looking to improve crop yields. Since biochar can alter soil N levels, it can alter leaf N content, in turn affecting photosynthesis (Xu et al., 2015). Zhu et al. (2014) noted that although biochar's positive effect on plant growth may be attributed to improved NUE, studies have shown contrasting results, possibly related to soil properties. Zheng et al. (2013) observed that biochar made from giant reed (*Arundo donax* L.) improved N bioavailability for maize growth in a silt loam, as observed in the higher NUE rates compared to the control. However, the giant reed biochar itself did not directly contribute much to soil N levels as it contained little available N, so the authors attributed the increased maize growth to higher soil microbial activity. Using seven different tropical and sub-tropical red soils, Zhu et al. (2014) saw an improvement in NUE in only two soils and suggested that the positive effect of biochar on maize growth was likely due to its liming effect.

While in our study there were neither significant differences between leaf N under the different treatments, nor a significant correlation between P_{plant} and leaf N, there were differences observed between biochars for NUE_{prod} and $PNUE_{plant}$. Cotton biochar had the lowest NUE_{prod} and $PNUE_{plant}$, and filtercake had the highest. Although available N was not measured in our study, when taking soil total N content into account, swine manure biochar had the highest soil N compared to the other biochars, and its NUE_{prod} was not significantly different from filtercake biochar. Since both biochars had lower C/N ratios than the other biochars (Table 3.1), they could potentially increase net mineralization and nitrification in the soil, increasing available N (Yoo and Kang, 2012). Eucalyptus biochar was not different from filtercake biochar, but it contributed to the lowest soil total N. Both filtercake and swine manure biochars may therefore have more potential to increase soil total N content, NUE, and PNUE in sandy soils compared to the other two biochars. Brantley et al. (2015) similarly suggested that combining biochar with N fertilizer could improve maize production based on increased NUE and yield compared to no biochar. Increased soil N retention through biochar could be due to increased N immobilization, reduced

N losses from gases and surface erosion, and higher organic N retention on biochar surfaces. In addition, biochar may increase N availability by providing substrate and habitat for microorganisms (Brantley et al., 2015).

Overall, the results of this study show that biochar combined with inorganic fertilizers can increase soil nutrient levels, but depending on the feedstock and application rate, it may lead to high pH and salinity that can negatively impact plant growth. PCA showed that increased $PNUE_{plant}$, P_{plant}, E_{plant}, g_{plant}, and Ca/Mg ratio correlated with increased dry aboveground biomass, plant height, and total LA for eucalyptus and filtercake biochars in particular, while R_{plant} and certain soil properties were negatively correlated with these plant physical properties especially for cotton biochars (Figure 3.8). Thus mixing biochars derived from different feedstocks (e.g. swine manure with filtercake) or mixing with raw feedstocks might enhance beneficial traits and reduce negative effects present in each feedstock individually (Lehmann and Joseph, 2015b).

3.5 Conclusions

In this greenhouse experiment, designed to evaluate the effect of different biochars on soil nutrients and plant physiology without nutrient or water limitations, maize plants had the greatest biomass on average in soils with filtercake biochar compared to the other biochars. It showed potential to increase maize biomass compared to the control and did not vary significantly with increasing application rate. Filtercake biochar also contributed to high soil Ca, Fe, and Mn levels, as well as the highest WUE_{prod}, NUE_{prod} and $PNUE_{plant}$ rates. In contrast, soils with cotton and swine manure biochars produced the lowest maize biomass, decreasing with increasing application rate. Yet, swine manure biochar is rich in nutrients and contributed to higher levels of CEC and certain soil nutrients (in particular, N), while cotton biochar can increase soil total C and shows potential for increasing soil water retention. Soils with eucalyptus biochar had high maize biomass, but overall eucalyptus biochar contributed little to soil fertility. It may contribute to soil structure through increasing soil total C. Our study highlighted the importance of the feedstock type application rates in order to maximize potential benefits. At higher applications rates, some biochar feedstocks may cause high salinity and/or possibly excessive water retention, affecting nutrient availability and plant physiology. Combinations of these biochars (e.g. cotton with filtercake or eucalyptus, or swine manure with filtercake) may help decrease risk of nutrient toxicity or deficiency (e.g. of K, S, Cu, Zn, Fe, Mn), while increasing C and N levels and maintaining adequate levels of AWC. Filtercake biochar in particular may increase crop production. These biochars show potential for addressing low water retention and soil fertility problems in Arenosols of the Brazilian Cerrado, when used in combination with inorganic fertilizers. If applied at proper rates, they offer a safe and beneficial alternative to disposing of farm waste readily available in the region.

Chapter 4: Dissolved organic carbon and nitrate in leachate as affected by biochar derived from agricultural waste materials: the influence of feedstock and pyrolysis temperature

4.1 Introduction

Arenosols (sandy soils) account for 13% of the area of the state of Mato Grosso, Brazil, (about 11.7 million ha), and their use as cultivated soils is increasing, particularly for growing maize (SEPLAN, 2008). However, Arenosols are low in organic matter, and their high sand content causes low water retention (da Costa et al., 2013). Carbon (C) in the form of dissolved organic carbon (DOC) and nutrients, such as nitrogen (N) in the form of nitrate (NO_3^-), are easily leached from these soils. As their use for agriculture is of increasing importance to the economy of Mato Grosso, sustainable management practices such as adding organic matter are necessary to improve the Arenosol's physico-chemical properties. Among the various types of organic amendments that can be added to soil, biochar is one that is considered efficient and stable in the long-term (Clough and Condron, 2010; Lehmann, 2007a). Biochar refers to charcoal derived from waste biomass by pyrolysis, which has been shown to improve fertility, carbon sequestration, and water-holding capacity in soils (Lehmann and Joseph, 2009). Its potential use in strategies for improving the agronomic performance of sandy soils in Mato Grosso could thus be beneficial. Less is known, however, about the effects of biochar use in tropical Arenosols, and more specifically, its role in retaining C and N in these soils.

Furthermore, examining the chemical reactivity of DOC can be useful to understand its contribution to ecosystem dynamics (Weishaar et al., 2003). Over the past few decades, fluorescence spectroscopy has proven to be a fast and relatively inexpensive method for characterizing DOC. Three-dimensional excitation-emission matrices (EEMs), produced by the combination of emission spectra with excitation wavelengths, can be used to produce fluorescence indices and intensities (Fellman et al., 2010). Several indices are used to determine different fluorescence characteristics. These include the fluorescence index (FI) which indicates whether the DOC is of terrestrial or microbial sources (Cory and Mcknight, 2005; McKnight et al., 2001), the biological index (BIX) described as "the index of recent autochthonous contribution" (p.716, Huguet et al., 2009), and the humification index (HIX), which measures the extent of humification (Zsolnay et al., 1999). Parallel factor (PARAFAC) analysis (Murphy et al., 2013) helps further characterize DOC composition (e.g. humic, protein-like, etc.) using data derived from EEMs (Fellman et al., 2010).

Besides its contribution to soil physical and chemical properties, biochar offers an alternative way to reduce agricultural waste compared to other organic amendments. Converting animal and crop waste to biochar significantly reduces the volume and weight of the waste, and requires fewer applications than fertilizers which need to be applied annually (Lehmann and Joseph, 2009). However, as Joseph et al. (2010) notes, the effect of biochar on the soil is "biochar- and site-specific". Thus in this study, a variety of agricultural wastes readily found in the region were transformed into biochar pyrolized at different temperatures to identify the influence of both feedstock and pyrolysis on the agroecological performance of biochar when applied to a Brazilian Arenosol. The objectives were to: 1) observe the effect of biochar type (feedstocks and temperatures of pyrolysis) on bulk leaching dynamics of DOC and NO_3^-, and 2) examine fluorescence characteristics of DOC leached from soil-biochar mixtures using fluorescence spectroscopy to infer DOC reactivity and fate. The hypotheses were that eucalyptus biochars would retain DOC more than the other biochars since it would increase soil C levels due to its feedstock’s high C/N ratio, while higher temperature biochars would reduce DOC losses particularly of humic DOC because of their greater recalcitrance.

4.2 Materials and methods

4.2.1 Soil collection and biochar production

Soils from the top 0-20 cm layer were collected from an agricultural field located within the farm Fazenda Água Azul (15°13'55.2"S, 54°57'43,4"W) managed by the agribusiness Grupo Bom Futuro, 178 km northwest of the state capital of Cuiabá in Mato Grosso, Brazil, an area within the Cerrado. The soil collected was classified as an Arenosol (FAO soil classification), with a sandy texture (91% sand, 4% silt, 5% clay). Carbon (C) and nitrogen (N) levels in the soil were 0.7 % C and 0.08 % N as determined by elemental analysis (628 Series CHN Analyzer, LECO Corp., St. Joseph, MI). The average $\mathrm{pH_{water}}$ was 5.8 and average CEC was 5.3 $\mathrm{cmol_c\ kg^{-1}}$, with a bulk density of 1.6 $\mathrm{g\ cm^{-3}}$. Over the last 10 years, the crops sown on the study site included soybean, sorghum, maize, and cotton, with the latter two crops grown in rotation with soy for the last three years (Afonso Campos da Silva, Grupo Bom Futuro, personal communication). Twelve biochars were commercially produced (SPPT Ltda., Mogi Morim, São Paulo, Brazil) from four feedstock materials: cotton husks, eucalyptus sawmill residue, sugarcane filtercake, and swine manure, slow-pyrolyzed at three temperatures (400°, 500°, 600°C). These were subsequently crushed and sieved to <2 mm in order to have similar biochar particle sizes between the different feedstocks and similar to soil particle size.

4.2.2 Experimental design

In a greenhouse located at the Federal University of Mato Grosso (UFMT), Cuiabá campus, 9 L volume pots with one hole drilled at the bottom were filled with 8 kg of an Arenosol. Twelve biochars (4 biochar feedstocks x 3 temperatures of pyrolysis) were applied to pots at 5% soil dry weight, mixed and compacted by hand, making a total of 52 pots (12 biochars x 4 replicates plus 4 unamended soil controls). A high biochar application rate (equivalent to 80t ha^{-1}) was used to ensure a biochar effect was detected. The pots were divided into 4 blocks, with each block running north-south along a greenhouse bench, with a replicate of each treatment (biochar amended soil) plus a control (unamended soil) randomly assigned to locations within each block. The greenhouse temperature was controlled to 28±2°C, similar to temperatures during which the dry season maize is grown from January to June (INPE, 2012).

Water was initially added to achieve field capacity and allowed to equilibrate. Since fertilizer applications are a standard management practice in the region, fertilizer was added to the pots corresponding to the amount each maize plant requires at the rate of 150 kg NPK+S, 150 kg KCl and 200 kg urea for 60,000 plants/hectare in the field (Afonso Campos da Silva, Grupo Bom Futuro, personal communication, 2014). Fertilizer, 2.5 g NPK+S (12-46-0 + 7), was added after 1 week and four maize seeds were planted in each pot. Crushed KCl (2.5g) and diluted urea (2.0 g in 50 mL water) were added 20 days after planting, followed by a second diluted urea application of 1.3 g 7 days later. Watering thereafter took place once a week then three times a week once the plants began to grow, adding water at 110% field capacity each time to produce sufficient leachate from each pot.

4.2.3 Laboratory analysis

Elemental analysis (C, H, N) of the 12 biochars were analyzed on a CHN Analyzer (628 Series, LECO Corp., St. Joseph, MI). Oxygen content of the biochars was calculated as O =100-(C+H+N+ash content). Ash content was determined by placing 1g of each biochar in crucibles and heating in a muffle furnace to 900°C for 4 h (Fuertes et al., 2010). Biochar properties are presented in Table 4.1. Total C and N in soils post-experiment were also analyzed on a CHN Analyzer.

Leachate was collected once per week for 6 weeks and filtered through 0.7 µm glass fiber filters. DOC and NO_3^- concentrations were determined weekly immediately after collection using a UV-Vis spectrophotometer (Spectrolyser; S-can, Austria) (Broeke et al., 2006). Since concentrations were higher than the spectrophotometer's range, samples were diluted with ultrapure water. DOC fluorescence characteristics from weekly samples were analyzed by obtaining EEMs on an Aqualog spectrofluorometer (Horiba Scientific, NJ, USA) as described by Eykelbosh et al. (2015). Briefly, samples were placed in a 10 mm quartz cuvette and analyzed at an integration time of 1 s. The fluorescence EEMs obtained from the Aqualog were then used to determine the fluorescence index (FI) (McKnight et al., 2001), humification index (HIX) (Zsolnay et al., 1999), and biological index (BIX) (Huguet et al., 2009), as well as PARAFAC components (Murphy et al., 2013).

The fluorescence index (FI) is derived from the ratio between emission (em) wavelengths at 450 and 500 nm at excitation (ex) wavelength 370 nm (McKnight et al., 2001). Low FI ratios (~1.4) indicate DOC primarily derived from terrestrial sources such as plants and soil organic matter, while higher FIs (~1.9) indicate DOC from microbial sources (McKnight et al., 2001). The BIX is calculated as the ratio between em wavelengths at 380 nm (representing the maximum intensity of the β fluorophore) and 430 nm (representing the maximum intensity of the α fluorophore) at 310 nm excitation (Huguet et al. 2009). Similar to the freshness index (β/α) (Parlanti et al., 2000), the β fluorophore is related to autochthonous and fresh DOM while the α fluorophore is associated with older, more degraded material. Higher ratios (>1) represent more recently produced DOM of biological origin, while lower ratios (0.6 – 0.7) correspond to more humified, less biological material (Birdwell and Valsaraj, 2010; Huguet et al., 2009). The HIX is calculated as the peak area under em 435 to 480 nm divided by em 300 to 445 nm, at ex 254 nm (Zsolnay et al., 1999). High ratios indicate higher humified organic material and thus the presence of more complex, aromatic molecules (Huguet et al., 2009). Low ratios (<10) indicate relatively non-humified DOM coming from biomass; as biomass decomposes, HIX ratios will increase (Birdwell and Valsaraj, 2010).

Table 4.1. Properties of 12 commercially-made biochars. Values shown are means±1 SE of three analytical replicates.

Biochar	**C (%)**	**N (%)**	**H (%)**	**O (%)**	**C:N**	**H:C**	**O:C**	**A.C.[a] (%)**
Cotton400	56.0±1.9	2.1±0.1	3.9±0.1	22.1±2.0	27.1±1.6	0.07±0.0	0.4±0.1	16.0±0.9
Cotton500	58.1±0.1	2.1±0.2	3.3±0.2	19.9±0.1	28.7±2.9	0.06±0.0	0.4±0.01	16.7±0.8
Cotton600	55.4±0.8	1.8±0.1	3.2±0.1	18.5±0.8	30.1±0.7	0.06±0.00	0.4±0.02	21.0±1.9
Swine400	36.7±0.3	2.9±0.01	2.6±0.7	12.4±0.6	12.8±0.1	0.07±0.01	0.3±0.01	45.4±1.6
Swine500	32.4±0.2	2.3±0.02	1.5±0.2	10.5±0.2	14.3±0.2	0.05±0.01	0.3±0.01	53.3±0.8
Swine600	31.7±0.3	1.9±0.0	1.3±0.3	7.3±0.5	16.6±0.1	0.04±0.02	0.2±0.01	57.8±0.2
Euca400	67.3±0.5	0.6±0.0	3.7±0.3	25.5±0.3	111.7±1.5	0.06±0.01	0.4±0.01	2.9±0.2
Euca500[b]	58.1±2.0	0.9±0.01	2.1±0.5	14.0±1.6	63.0±1.6	0.04±0.01	0.2±0.03	24.9±1.6
Euca600	73.9±0.9	0.7±0.01	3.6±0.06	17.0±0.9	106.0±1.1	0.04±0.00	0.2±0.01	4.7±0.5
Filter400	19.3±0.4	1.6±0.04	3.4±0.04	11.8±0.5	12.3±0.04	0.2±0.0	0.8±0.04	64.0±0.6
Filter500	19.6±0.8	1.6±0.1	2.4±0.03	4.8±0.8	12.0±0.02	0.1±0.0	0.4±0.1	71.6±0.5
Filter600	23.3±1.2	1.8±0.1	1.7±0.04	0.0±0.0	12.8±0.2	0.1±0.0	0.0±0.1	74.7±1.0

[a] A.C. = ash content

[b] Euca500 may have mixed with another feedstock during biochar production and therefore has higher ash content than Euca400 and 600.

4.2.4 Statistical analysis

The effects of biochar treatments including the control on DOC and NO_3^- concentrations were determined by repeated measures analysis of variance (ANOVA) using IBM® SPSS® Statistics (Version 23, SPSS. Inc., Chicago, USA), with "treatment" as the between-subjects factor and "time" as the within-subjects factor. Treatments were then separated by biochar feedstock and temperature of pyrolysis and their effect on total DOC and NO_3^- concentrations was determined by multivariate ANOVA (MANOVA). Where differences were significant, a post-hoc Games-Howell test ($P < 0.05$) was used to compare means, as variances were unequal. Pearson correlation coefficient and linear regression between DOC and NO_3^- were determined. Values presented in graphs and text are means ±1 standard error (SE).

EEMS data was corrected for inner filter effects, dilution factors, and Raman scattering, before performing Raman normalisation, on R (version 3.3.1) using RStudio. Fluorescence indices (FI, HIX, and BIX) were also determined by R following calculations by McKnight et al. (2001), Zsolnay et al. (1999), and Huguet et al. (2009) for each index, respectively, which was performed using the eemR package for R (Massicotte, 2016). PARAFAC modeling with non-negativity constraint was carried out using MATLAB (R2016a, The MathWorks Inc., USA) and the drEEM 0.1.0 and N-way 3.20 toolboxes, to determine the number of components in the model following the tutorial by Murphy et al. (2013). Split-half analysis was used to validate the PARAFAC model (Murphy et al., 2013). The type of fluorescence component and its probable source was then described following Fellman et al. (2010). The fluorescence intensity at the maximum (Fmax) (Murphy et al., 2013) was also determined by PARAFAC analysis for each component and sample. The effect of biochar treatments on fluorescence indices and on Fmaxes over time were determined with repeated measures ANOVA on SPSS. Relative abundance (%)

of Fmaxes was calculated as Fmax/ΣFmax (Murphy et al., 2013) and differences between abundances tested by one-way ANOVA for each treatment. Where differences between treatments were significant, but variances unequal, a post-hoc Games-Howell test ($P < 0.05$) was used to compare means. Pearson correlation coefficients and linear regressions were determined between soil C/N ratios and DOC and NO_3^- concentrations with fluorescence indices and Fmaxs. Principal component analysis (PCA) on DOC and NO_3^- concentrations and DOC characteristics (FI, BIX, HIX, Fmaxs, and C/N ratio) in biochar treatments was carried out with the FactoMineR package (Le et al., 2008) in R (version 3.3.1).

4.3 Results

4.3.1 DOC and NO_3^- leaching varies among biochars

The repeated measures ANOVA for DOC and NO_3^- concentrations showed that there was a significant treatment, time, and time*treatment effect (Figure 4.1). Observing concentrations over time, leachate from cotton and swine manure biochars contained very high levels of DOC and NO_3^- in the first few weeks, but leveled off by the sixth week, whereas DOC and NO_3^- in leachate from filtercake and eucalyptus remained relatively stable and similar to the control throughout the 6 weeks (Figure 4.1A and B). There was a slight increase in NO_3^- concentrations in weeks 5 and 6 for all treatments except soils with cotton biochar. This increase is likely related to the urea applications in weeks 4 and 5.

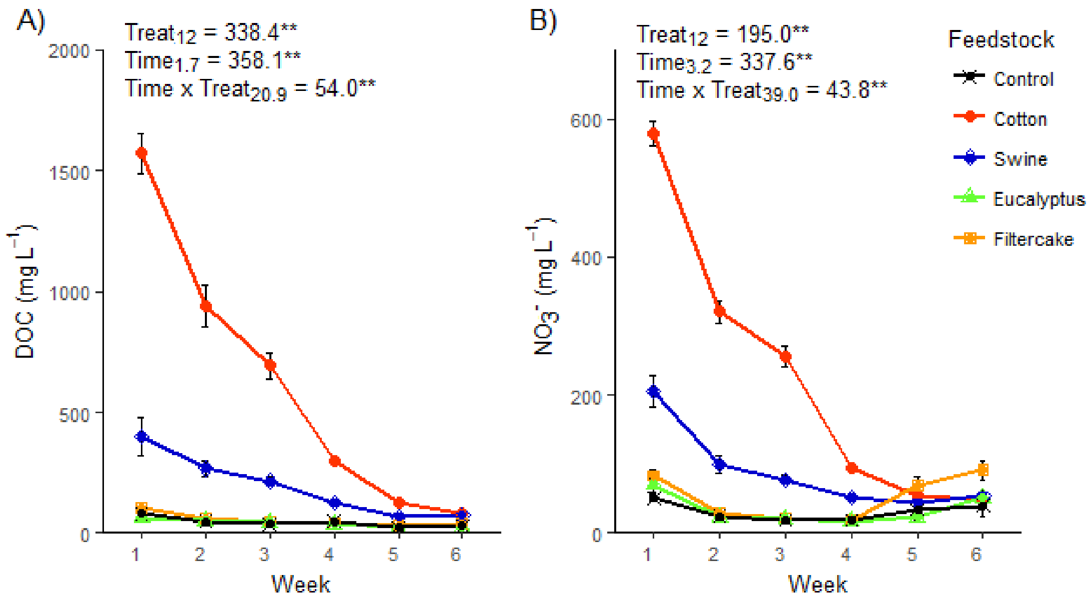

Figure 4.1. A) Mean dissolved organic carbon (DOC, mg L^{-1}) and B) nitrate (NO$_3^-$, N mg L^{-1}) per week per biochar feedstock and control, with results of the repeated measures ANOVA for the effect of biochar treatment (Treat) on weekly (Time) DOC and NO$_3^-$ measurements. A Greenhouse-Geisser correction was applied for effect of time and its interaction to account for lack of sphericity. ** = *P* <0.01.

When the biochar treatments were separated by feedstock and temperature of pyrolysis, the type of feedstock had a significant effect on total DOC and NO$_3^-$ levels, while there was no significant temperature effect (Figure 4.2). However, there were temperature differences for each feedstock except cotton biochar (Figure 4.2A). Leachate from soils with cotton biochars had the greatest mean DOC (618.4±557.3 mg L^{-1}) over the experiment, followed by swine manure (190.8±172.4 mg L^{-1}), filtercake (51.9±46.3 mg L^{-1}) and eucalyptus (40.8±19 mg L^{-1}) biochars. Mean DOC values for filtercake and eucalyptus did not differ from each other nor were significantly greater than the control (44.6±23.5 mg L^{-1}) (Figure 4.2A). Mean NO$_3^-$ had similar treatment effects as DOC, with only swine manure biochar showing a difference between the temperatures (Figure 4.2B). DOC and NO$_3^-$ concentrations in leachate were significantly ($P < 0.05$) correlated, with an

R^2 of 0.91. In addition, all biochars significantly ($P < 0.05$) increased total soil C (%) compared to the control, with filtercake biochars increasing soil C the least (Figure 4.3A). All biochars, except for eucalyptus biochar, also significantly increased total soil N (%) compared to the control (Figure 4.3B). There were no differences between the temperatures for either soil C or N.

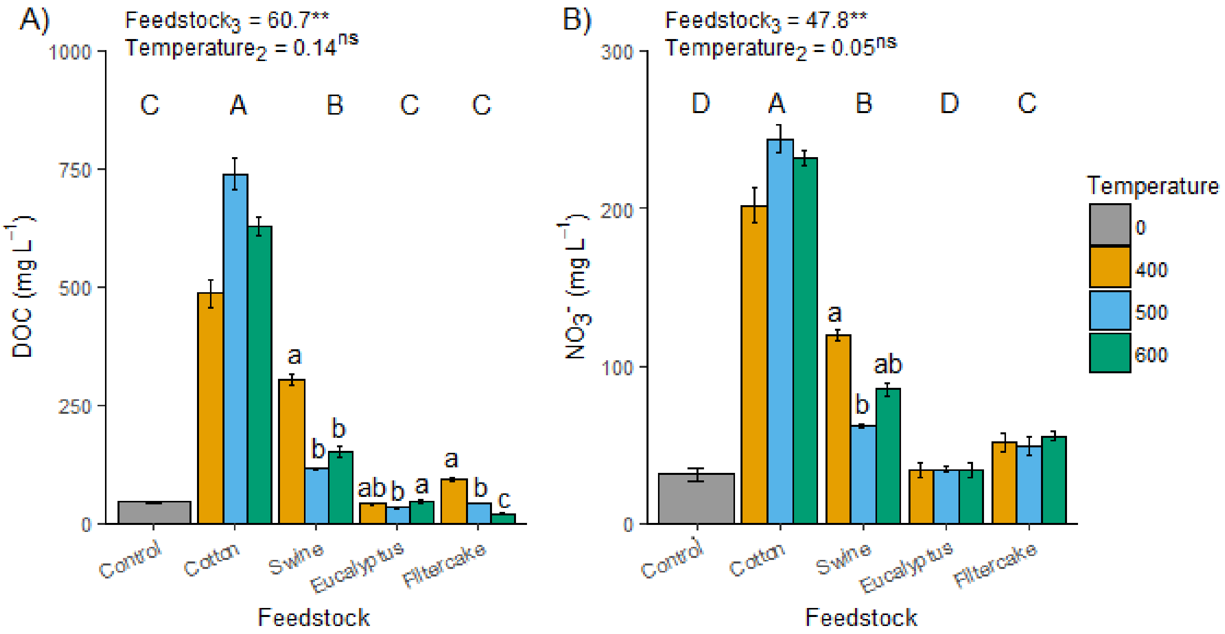

Figure 4.2. A) Mean dissolved organic C (DOC, mg L^{-1}) and B) nitrate (NO_3^-, mg L^{-1}) per biochar feedstock and temperature of pyrolysis over 6 weeks (mean±1 SE) with results of the MANOVA. ** = *P*<0.01, ns = not significant. Capital letters indicate significant differences between the feedstocks and control and lowercase letters, between temperatures for each feedstock (Games-Howell test; *P*<0.05).

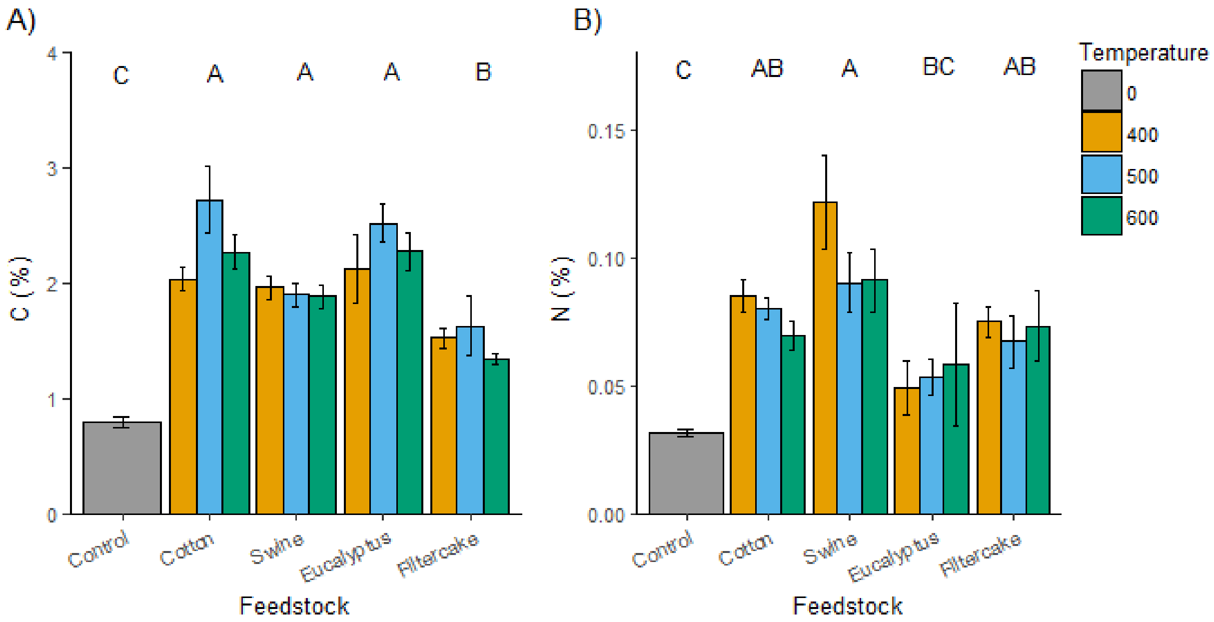

Figure 4.3. A) Mean soil total C (%) and B) total N (%) per biochar feedstock and temperature of pyrolysis after 6 weeks. Capital letters indicate significant differences between the feedstocks and control (Games-Howell test; *P* <0.05).

4.3.2 DOC fluorescence indices

The repeated measures ANOVA for the fluorescence indices showed a significant treatment, time, and time*treatment effect for all three indices (Figure 4.4). Looking at changes over time, for both FI and BIX, almost all treatments including control experienced a peak in week 5 (Figure 4.4A and B), similar to the increase observed in NO_3^- concentrations. However, neither was significantly correlated with NO_3^- concentrations. FI was very poorly, but significantly, correlated with DOC concentrations (R = 0.14; $P < 0.05$). For HIX, the opposite was observed, with a drop in week 5 for most treatments (Figure 4.4C). HIX was significantly, but also very poorly, correlated with NO_3^- concentrations (R = 0.14; $P < 0.05$), as well as with DOC concentrations (R = 0.17; $P < 0.01$). Although the correlations were not significant, higher soil C/N ratios tended to have low FI and BIX values and high HIX values (Appendix Figure H.1).

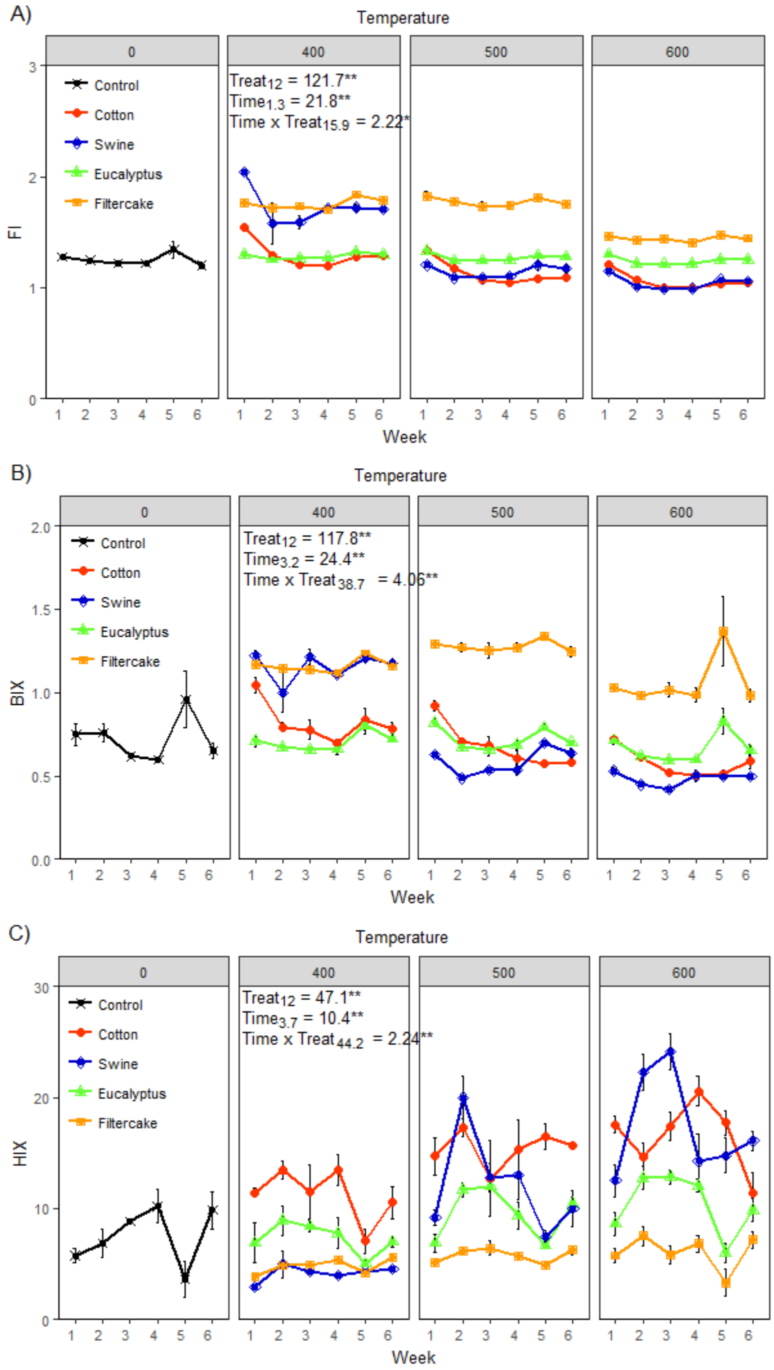

Figure 4.4. Mean weekly A) fluorescence index (FI), B) biological index (BIX), and C) humification index (HIX) (no units) per biochar feedstock and temperature as well as control, with results of the repeated measures ANOVA for the effect of biochar treatment (Treat) on weekly (Time) DOC characteristics. A Greenhouse-Geisser correction was applied for effect of time and its interactions to account for lack of sphericity (*=*P*<0.05, ** = *P*<0.01).

When looking at the biochar feedstocks and temperatures of pyrolysis, cotton biochars had the lowest FIs, followed by eucalyptus and swine manure biochars. Filtercake biochar had the highest FIs and was the only biochar with FIs significantly higher than the control (Figure 4.5A). FIs decreased significantly ($P < 0.05$) as temperature of pyrolysis increased for all biochars, although the 400 and 500°C did not differ significantly for the eucalyptus and filtercake biochars (Figure 4.5A). For the BIX, cotton, eucalyptus and swine manure biochars were not significantly different from each other, while filtercake biochar had the highest BIXs compared to the other biochars and the control. The differences between temperatures of pyrolysis were similar as the FIs for cotton and swine manure biochars, with BIX decreasing with increasing temperature. The BIX for eucalyptus, however, did not differ between temperatures and for filtercake, the 400°C temperature had higher mean BIX than the 600°C, but was not higher than the 500°C (Figure 4.5B). For the HIX, cotton, swine manure, and filtercake biochars were significantly ($P < 0.05$) different from each other, but swine manure biochar was not significantly different from eucalyptus biochar which did not differ from the control. Only cotton and swine manure biochars had significantly higher HIX than the control. The differences between the temperatures showed the opposite trend seen with the FI and BIX: the HIX increased with increasing temperature of pyrolysis. Although the HIX for the 500 and 600°C temperatures did not always vary from each other (except for swine manure), the HIX for the 400°C was always lower than the 600°C temperature (Figure 4.5C).

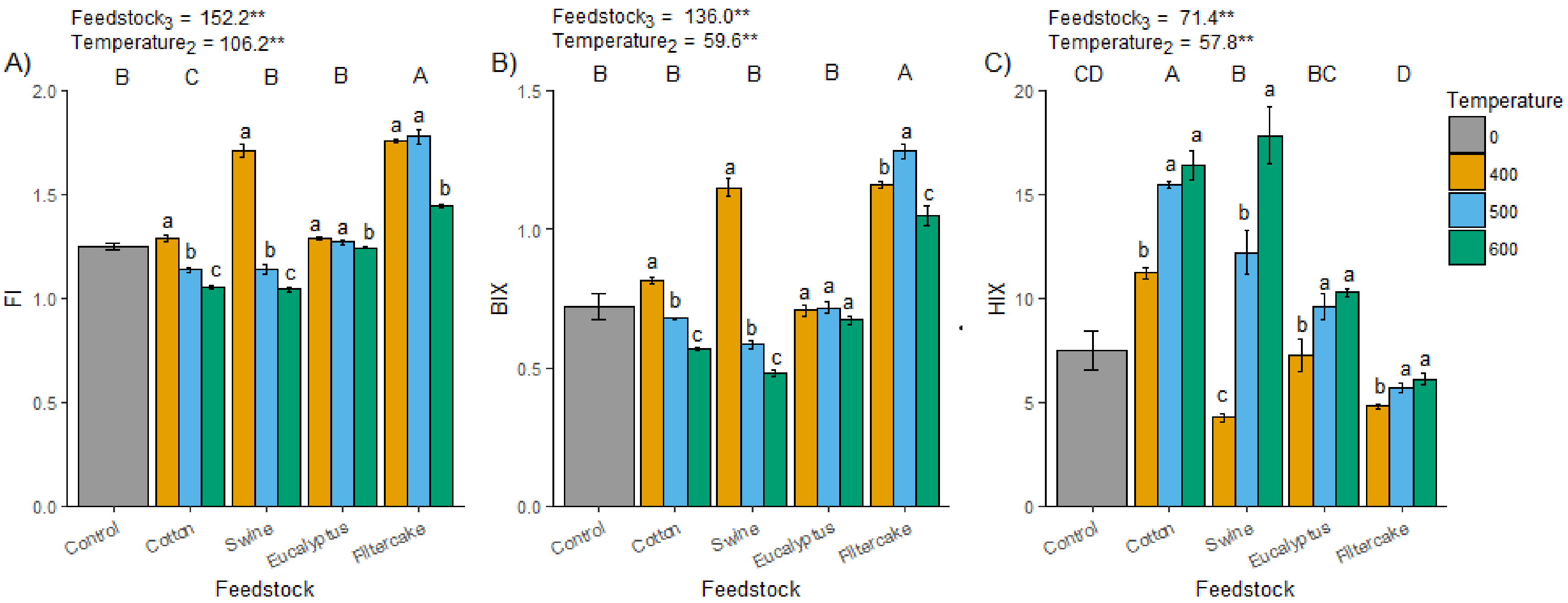

Figure 4.5. Mean A) fluorescence index (FI), B) biological index (BIX), and C) humification index (HIX) (no units) per biochar feedstock and temperature of pyrolysis after 6 weeks, with results of the MANOVA. ** = *P*<0.01. Capital letters indicate significant differences between the feedstocks and control and lowercase letters indicate significant differences between temperatures for each feedstock (Games-Howell test: *P* <0.05).

4.3.3 EEMs PARAFAC analysis

Comparing models with 2- to 8-components through PARAFAC analysis of EEMs, a 5-component model was applied based on split-half validation and PARAFAC results of other biochar or soil DOC studies (e.g. Uchimiya et al., 2013). Examples of EEMs for each treatment in our study are shown in Appendix Figures I.1 and I.2, the spectral loadings and contour plots of the 5-component model in Appendix Figure I.3.

Based on the PARAFAC analysis and following the review by Fellman et al. (2010), the 5 components identified were: 1) UVC humic-like, 2) UVA humic-like, 3) UVC humic-like, 4) humic-like, and 5) tryptophan-like, summarized in Table 4.2. The mean and standard error of the Fmax (Raman units) of each component for each treatment are shown in Appendix Table E.1. Overall, components 1 and 2 had higher mean Fmaxs compared to components 3, 4, and 5.

Table 4.2. PARAFAC components identified following the review by Fellman et al. (2010)

Component	Excitation (nm)	Emission (nm)	Source and description
1) UVC humic-like	320-360	420-460	Terrestrial; high-molecular-weight humic
2) UVA humic-like	290-325 (<250)	370-430	Likely derived from autochthonous production or microbial processing; low molecular weight
3) UVC humic-like	<250 (305)	412-416	Terrestrial; high molecular weight humic
4) humic-like	250	550	Terrestrial or microbial sources; reduced humic-like
5) tryptophan-like	270-280 (<240)	330-368	Terrestrial, autochthonous production,

Component	Excitation (nm)	Emission (nm)	Source and description
			or microbial processing; may reflect intact protein or partially degraded peptides

Separating the treatments by feedstock and temperature for each component's Fmax (Figure 4.6) showed that there were differences between feedstocks and temperatures of pyrolysis for some feedstocks. For Fmax1 (UVC humic-like), cotton, swine manure, and filtercake biochars were significantly (*P*<0.05) different from the control and eucalyptus biochar. For swine manure and filtercake feedstocks, the biochars pyrolysed at 400°C had higher Fmaxs than at 500 and 600°C (Figure 4.6A). For Fmax2 (UVA humic-like), swine manure and filtercake feedstocks were significantly greater than the control and the other two feedstocks. For cotton, swine manure and filtercake biochars, the 400°C biochars had the highest mean Fmaxs compared to the other temperatures, although it was not significantly different than the 500°C biochar for filtercake (Figure 4.6B). For Fmax3 (UVC humic-like), all biochar feedstocks were significantly different from each other (*P*<0.05), but only cotton and swine manure biochars were different from the control. Only swine manure and filtercake biochars had differences between the temperatures, with the Fmax for 400°C lower than that of 600°C (Figure 4.6C). For Fmax4 (humic-like), cotton and swine manure biochars had significantly greater Fmaxs than the control and the other biochars. For cotton, the 400°C biochar had significantly lower Fmax than the 500°C, for swine manure 400°C was significantly greater than the 500°C, and for filtercake the 400°C was significantly greater than the 600°C (Figure 4.6D). Lastly, for Fmax5 (tryptophan-like), cotton, swine manure, and filtercake biochars all had significantly greater Fmaxs than the control and eucalyptus biochar. Between the temperatures, the swine manure 400°C biochar was significantly greater than both the 500 and 600°C, and the filtercake biochar followed the pattern 400 > 500 > 600°C (Figure 4.6E). None of the Fmaxs were correlated with DOC or NO_3^- concentrations.

For the control, its fluorescence was mostly represented by Component 1 (29%) compared to the other components. Component 3 was most abundant for cotton 400 (37%), 500 (43%), and 600°C (38%) and swine manure 500 (28%) and 600°C (32%) biochars, but for swine manure 400°C biochar Component 2 was greatest (45%). The eucalyptus biochars were similar to the control with the Component 1 most abundant, while filtercake biochars had Component 2 dominating. Component 4 was the second most dominating for cotton biochars and swine manure 500 and 600°C biochars, while Component 5 was also high for swine manure 400°C and the filtercake biochars (Table 4.3 and Appendix Figure I.4).

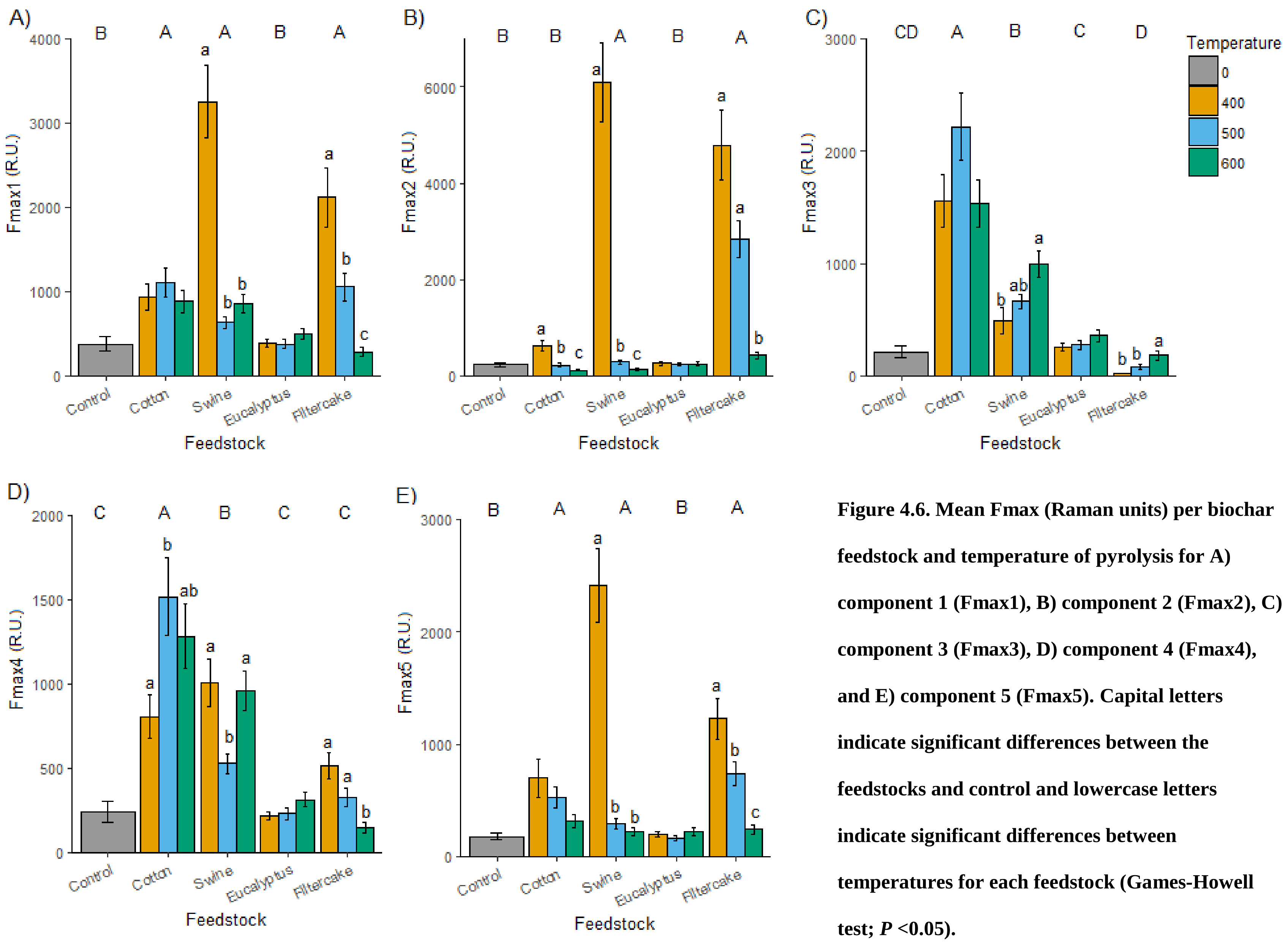

Figure 4.6. Mean Fmax (Raman units) per biochar feedstock and temperature of pyrolysis for A) component 1 (Fmax1), B) component 2 (Fmax2), C) component 3 (Fmax3), D) component 4 (Fmax4), and E) component 5 (Fmax5). Capital letters indicate significant differences between the feedstocks and control and lowercase letters indicate significant differences between temperatures for each feedstock (Games-Howell test; *P* <0.05).

Table 4.3. Percent relative abundance (Fmax/ΣFmax) of each component Fmax for a PARAFAC 5-component model for biochar treatments and control (mean ± 1 SE).

	Fmax relative abundance (%)				
Biochar	1	2	3	4	5
Control	29.4±0.9	19.5±0.55	16.6±0.8	17.5±0.9	17.0±2.1
Cotton400	19.3±0.6	13.4±0.5	36.6±1.3	16.9±0.8	13.8±1.4
Cotton500	18.3±0.6	4.4±0.3	42.5±1.2	24.7±1.0	10.0±1.0
Cotton600	20.8±0.3	3.1±0.3	38.4±0.6	30.0±0.8	8.1±0.8
Swine400	24.2±0.2	45.3±2.2	4.4±1.2	7.8±1.0	18.3±0.5
Swine500	26.4±0.4	12.0±0.9	28.1±0.7	21.6±0.9	11.9±1.1
Swine600	27.3±0.4	4.7±0.4	31.5±0.3	29.1±0.8	7.4±0.9
Eucalyptus400	29.6±0.4	19.9±0.5	19.2±0.6	16.3±0.4	15.1±0.9
Eucalyptus500	29.0±0.4	19.6±0.8	20.5±0.6	17.3±0.5	13.6±0.8
Eucalyptus600	30.4±0.7	16.8±0.8	20.8±0.7	18.8±0.5	13.2±1.5
Filtercake400	23.3±0.5	56.0±0.7	0.8±0.4	5.8±0.2	14.1±0.4
Filtercake500	19.7±0.7	58.2±1.3	1.5±0.3	6.2±0.2	14.3±0.4
Filtercake600	21.9±0.5	34.5±0.8	13.5±0.5	11.1±0.3	19.1±1.5

4.3.4 Principal component analysis

The PCA on DOC and NO_3^- concentrations along with DOC characteristics (fluorescence indices and PARAFAC components) showed several groupings for each of the four biochar feedstocks (Figure 4.7). Cotton biochars had three groupings: FI, BIX, Fmax2, and Fmax5 (Group 1), Fmax1 and Fmax3 (Group 2), and Fmax4, DOC concentration, NO_3^- concentration, C/N ratio, and HIX (Group 3). The cotton biochars at 400°C clustered near Group 1, while the 500°C biochars clustered closer to Group 2 and the 600°C biochars to Group 3 (Figure 4.7A). For the swine manure biochars, the PCA showed three groupings: Fmax4, Fmax3, and NO_3^- concentration (Group 1), BIX and FI (Group 2), and DOC concentration, C/N ratio, Fmax1, Fmax2, Fmax5, and HIX (Group 3). Swine manure biochars at 400°C clustered closely to Group 2, the 500°C biochars to Group 1, and the 600°C biochars to Group 3 (Figure 4.7B). For eucalyptus biochars, two groupings stood out: HIX, DOC and NO_3^- concentrations, and C/N ratio (Group 1), and Fmax1, Fmax2, Fmax3, Fmax4, Fmax5, FI, and BIX (Group 2). Eucalyptus biochars at 400°C and 500°C clustered closely to Group 1, while the 600°C biochars clustered by Group 2 (Figure 4.7C). Lastly, the PCA for filtercake biochars showed two main groupings: Fmax3 and DOC concentrations (Group 1) and BIX, FI, NO_3^- concentrations, C/N ratios, Fmax1, Fmax2, Fmax3, Fmax4, Fmax5, and HIX (Group2). Filtercake biochars 400°C clustered closely to Group 2, while the 500°C and 600°C biochars clustered closer to Group 1 (Figure 4.7D)

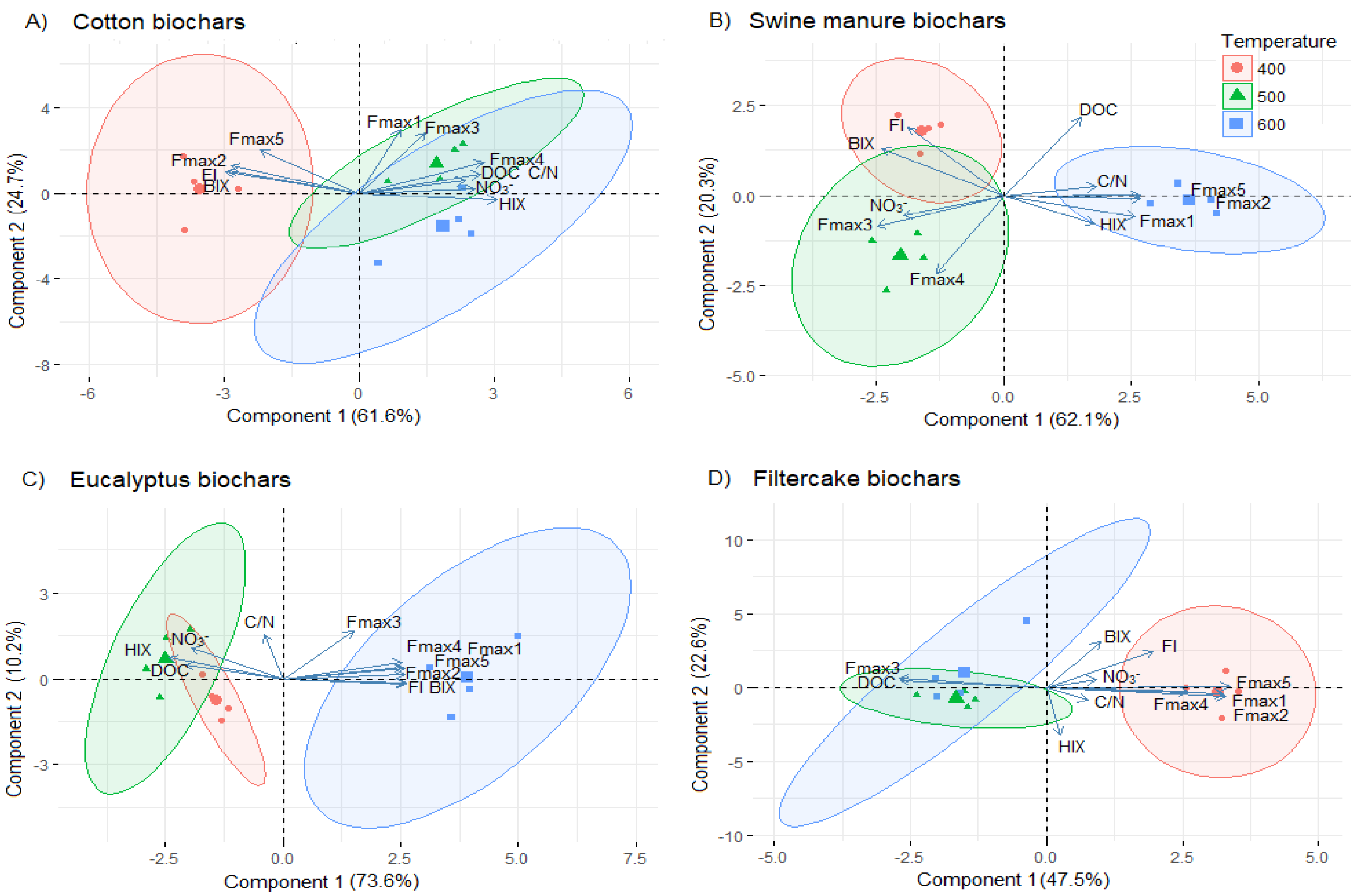

Figure 4.7. Principal component analysis (PCA) of DOC and NO_3^- concentrations and DOC characteristics from soils with 12 biochars (4 feedstocks x 3 temperatures of pyrolysis).

4.4 Discussion

4.4.1 DOC leaching

Cotton and swine manure biochars led to much higher DOC losses in soil leachate compared to the control soils at the initial time of application. During biochar pyrolysis, low- molecular-weight organic compounds are produced that are labile or leachable, and some can adsorb to the biochar surface (Lin et al., 2012). The high losses from cotton and swine manure biochar treatments after initial application are likely derived from the more labile polysaccharide organic matter of the biochars which do not adsorb as well and are flushed into the DOM (Kaiser and Guggenberger, 2000). Barnes et al. (2014) observed a similar effect when biochar was added to sandy soils with low organic matter, suggesting that the C source in DOC losses was mostly biochar-derived, and not from the soil itself. As in our study, DOC losses from biochar-amended soils in Barnes et al. (2014) decreased over time, implying that the more labile biochar-C was rapidly depleted.

In contrast to the cotton and swine manure biochars, eucalyptus and filtercake biochars did not have drastically higher initial peaks in DOC leaching, but rather remained relatively stable throughout the six weeks. This suggests that the eucalyptus and filtercake feedstocks have less labile or leachable C than the cotton and swine manure feedstocks. Similarly, Eykelbosh et al. (2015) observed that sugarcane filtercake biochar decreased DOC export in a 4-month column experiment and that the leached DOC consisted mostly of labile components. Cotton biochar and eucalyptus biochar had high levels of C compared to the other biochars for all pyrolysis temperatures (Table 4.1), but C from cotton biochar appears to have been more labile. This is probably due to the high lignin content in eucalyptus, which ranges between 23% to 34% (Rodrigues et al., 1998), compared to lower lignin content in cotton, around 15% (Ververis et al., 2004). Sugarcane filtercake, similar to eucalyptus, has a relatively high lignin content which can be around 32% (Eykelbosh et al., 2014). Animal manures typically contain lower lignin contents (Brown et al., 2015), noting that pig feed in Brazil mostly consists of cereals such as low-lignin content maize and rice, and legumes such as soybean (EMBRAPA, 2003), thus containing more labile C from cellulose and hemicellulose than woody feedstocks or sugarcane.

H/C and O/C ratios are often used as measures of aromaticity levels in biochar, with higher H/C and O/C ratios observed for low-temperature biochars (Krull et al., 2009). This was the case for our biochars where the H/C and O/C ratios were higher for the 400°C biochars compared to the 500 and 600°C biochars (Table 4.1). Low H/C and O/C ratios imply higher aromaticity and stability (Keiluweit et al., 2010; Van Zwieten et al., 2010). As temperature of pyrolysis increases, aromatic C in biochars increases, with biochars produced at temperatures $\geq$ 400°C containing less than 10% non-aromatic C (Kleber et al., 2015). Differences between temperatures of pyrolysis were observed for DOC leached from soils with swine manure, eucalyptus, and filtercake biochars (Figure 4.2A). The biochars at 400°C had significantly greater DOC losses than the higher temperatures for swine manure and filtercake feedstocks, but not for eucalyptus. The higher non-aromatic fraction in low-temperature biochars may make them more accessible for microbial activities, e.g. decomposition, compared to high-temperature biochars (Joseph et al., 2010).

Cotton and swine manure biochars, particularly at lower temperatures as noted for swine manure biochar, may have stimulated microbial activity and SOM decomposition more, leading to higher DOC leaching than the other biochar feedstocks. Yet, biochar amendment can also decrease soil respiration and thus SOM decomposition (Eykelbosh et al., 2015; Jones et al., 2011; Keith et al., 2011), which may have occurred in eucalyptus and filtercake biochar treatments. DOC concentrations in leachate lowered over the course of the experiment (Figure 4.1A), suggesting that microbial activity stabilized as the biochar-soil mixtures aged. These results imply that feedstock played a greater role in retaining DOC in the soil compared to pyrolysis temperature.

4.4.2 NO_3^- leaching

Cotton and swine manure biochars contributed to higher NO_3^- losses in their leachate compared to the control and the other two biochars. Manure-based biochars often have high total N content because of the high protein content of their feedstock. Plant-based biochars in turn usually have less N, but higher C content (Ippolito et al., 2015). Both cotton and eucalyptus biochars had high total C, but unlike eucalyptus biochar, cotton biochar had high total N similar to swine manure biochar and greater than filtercake biochar (Table 4.1). However, available N in biochars in the form of NO_3^- has been reported in the literature to be mostly negligible (Ippolito et al., 2015). Thus, cotton and swine manure biochars led to release of N from the soil as NO_3^- while eucalyptus and filtercake biochars retained NO_3^- in the soil, but not significantly more than the control.

Our results are in contrast to other studies, such as Uzoma et al. (2011b) who observed that black locust biochar significantly retained NO_3^- in sandy soils compared to the control over time. Zheng et al. (2013) also found that giant reed biochar reduced NO_3^- leaching after N fertilizer application. Biochar has the potential to adsorb ammonia (NH_3), as well as retain ammonium (NH_4^+) by increasing CEC, thus reducing nitrification and preventing NO_3^- leaching (Clough and Condron, 2010).

The high initial NO_3^- losses in our study are likely due to the NPK fertilizer application in the first week, and the increase in weeks 5 and 6 to the urea applications in weeks 4 and 5, as the recent N inputs may have stimulated microbial activity. The differences between the biochar treatments, however, may be related to increased nitrification in the soils, with cotton and swine manure biochars causing more nitrification than the other biochars. Eykelbosh et al. (2015) also noted increased NO_3^- leaching in filtercake biochar-amended soils, suggesting the biochar may have increased mineralization of soil organic N by improving soil porosity and aeration.

Nitrification in soils is related to NH_4^+ availability; if NH_4^+ adsorbed to biochar remains available, soil NO_3^- levels, and presumably leaching, would increase in soils with biochars (Thies et al., 2015). Dempster et al. (2012) observed a significantly reduced inorganic N pool in soils with Jarrah wood (*Eucalyptus* sp.) biochar, as well as decreased nitrification rate with increasing biochar application rate in all three N treatments (organic N, inorganic N, and basal N additions). The authors suggested the reduced nitrification rate in the presence of biochar was due to lower NH_4^+ levels caused by substrate limitation; biochar had a negative effect on SOM decomposition as well. The opposite may have occurred in our study: cotton and swine manure biochars provided additional microbial substrate, contributing to increased NH_4^+ levels in the soils and increasing nitrifying activity and NO_3^- production. In fact, Yoo and Kang (2012) found that swine manure biochar increased net N mineralization and net nitrification in silt loam soils in a laboratory incubation study, stating a need for caution when using high N biochars as soil amendments. Eucalyptus and filtercake biochar, in contrast, may not have contributed as much to NH_4^+ levels in the soil. Although biochars with high C/N ratio (>30) can cause lower N mineralization, this can be overcome by adding N fertilizer (Jeffery et al., 2015a), as was done in the present study. In addition, cotton biochar (with a high C/N ratio) and filtercake biochar (with a low C/N ratio) had the opposite effects on NO_3^- leaching, and NO_3^- concentrations were not significantly correlated with soil C/N ratios. Other mechanisms that may have affected higher

NO_3^- losses in cotton and swine manure biochar treatments are increased hydraulic conductivity (Kameyama et al., 2012) and increased negative charge density (Liang et al., 2006) in soils amended with these biochars.

4.4.3 DOC characteristics

4.4.3.1 Fluorescence Index

Over time, FI decreased for most treatments (Figure 4.4A), indicating that DOC derived from microbial sources decreased as microbial activity slowed down. At week 5, however, DOC from all biochars experienced an increase in FI suggesting an increase in microbial activity. Although there was no significant correlation between mean FIs and NO_3^-, the FI peaks may be related to the NO_3^- increases also observed in week 5 following urea additions the week before, which may have stimulated microbial activity. Despite DOC and NO_3^- being highly correlated in our study, and evidence of a strong link between the C and N cycles (Grant, 1995), no relationship between FI (or other DOM characteristics) and NO_3^- was found, as was the case for Tye and Lapworth (2016). The FI was also overall independent of DOC concentrations in our experiment, as noted in other studies (e.g. Jaffé et al., 2008; Tye and Lapworth, 2016).

In our study, all treatments had mean FIs between 1.2 and 1.4 except for swine manure biochar at 400°C (1.7±0.04), and filtercake biochar at 400 (1.8±0.01) and 500°C (1.8±0.02) (Figure 4.5¡Error! La autoreferencia al marcador no es válida.A). This suggests that DOC from all biochar treatments was mainly derived from terrestrial sources, but DOC from swine manure at 400°C and filtercake at 400 and 500°C had more microbial sources than the other biochar treatments. High FI values, which indicate more microbially-sourced DOC, are due to a decline in emission with increasing wavelengths in samples with microbial DOC (McKnight et al., 2001), while for terrestrial DOC, emission intensity increases with increasing wavelengths. DOC derived from terrestrial sources usually contains more lignin than microbially-derived DOC (Fellman et al., 2009); thus a higher FI at a low temperature of pyrolysis would be consistent for swine manure biochar. In contrast, filtercake has a relatively high lignin content (Eykelbosh et al., 2014) which does not explain the high FI for DOC from our treatments with filtercake biochars at 400 and 500°C. Fresh filtercake additions, however, can stimulate soil microbial activity and respiration (Rasul and Khan, 2008). The filtercake biochars at 400 and 500°C may therefore still have contained enough bioavailable C to contribute to soil microbial activity compared to filtercake biochar at 600°C, since non-labile C fractions increase with increasing temperature of pyrolysis (Nelissen et al., 2012). Similarly, in a study comparing DOM from different soil types, Tye and Lapworth (2016) noted through principal component analysis that the DOM from the soils was mostly terrestrially derived, and that the labile components were likely related to microbial activity. In addition, although not significant, FI values in our study tended to increase with lower soil C/N ratios, suggesting that biochar treatments with low soil C/N ratios had DOC derived from mostly microbial, rather than terrestrial, biomass (Jaffé et al., 2008). Both filtercake and swine manure biochars had relatively low C/N ratios compared to cotton and eucalyptus biochars (Table 4.1).

4.4.3.2 Biological Index

The BIX in our study varied significantly over time, in general lowering in the first few weeks with a peak occurring for most treatments in week 5 (Figure 4.4B). Organic material in the treatments was thus decomposing in the first few weeks before a nutrient input (e.g. urea) caused an increase in microbial activity resulting in the release of more autochthonous, fresh DOC. In our study, cotton, swine manure, and eucalyptus biochars did not differ significantly from each other or from the control; only filtercake biochar had significantly greater BIX than the other treatments (Figure 4.5B). This suggests that the DOC leached from filtercake biochar treatments was fresher than that lost from the other biochar treatments. Swine manure biochar at 400°C also had similar BIX as the filtercake biochars at the lower temperatures, a similar trend as with their FIs. Although not significantly correlated, treatments with lower soil C/N ratio had higher BIX values (Appendix Figure H.1), indicating DOC from those treatments was fresher. Not surprisingly, for most treatments, the BIX decreased with increasing temperature of pyrolysis, as biochar becomes more resistant to decomposition at higher temperatures (Kleber et al., 2015).

4.4.3.3 Humification Index

The HIX varied considerably over the time of the experiment, increasing in the first two weeks, then dropping either gradually or dramatically for some treatments until the lowest point at week 5 (Figure 4.4C). The HIX for cotton biochar at 400 and 500°C, however, increased from week 3 before gradually dropping towards the end of the experiment at week 6. DOM in most treatments thus began as less humified, indicating microbial activity, followed by more humified as decomposition progressed and slowed down, and then experienced a burst of microbial activity (again possibly due to urea applications) which once again lowered humification rate. This variation over time is not unusual as DOM quantity and quality are known to vary spatially and temporally in relation to its source material and environment (Hansen et al., 2016). Birdwell and Valsaraj (2010) observed that DOM in fogwater samples changed significantly from more humified (HIX 6.4) and terrestrially sourced (FI 1.4, BIX 0.6) to less humified (HIX 3.9) and biologically sourced (FI 1.8, BIX 0.1) in only a 4h period. As with the FI and BIX for our treatments, the HIX was not significantly correlated with the soil C/N ratio, but a trend was noticeable of higher HIX with higher soil C/N ratio.

HIX values from soils can range from 10 to 30 (Birdwell and Engel, 2010). In our study, HIX values ranged from as low as 4.3 (swine manure biochar at 400°C) to as high as 17.5 (swine manure biochar at 600°C). Cotton and swine manure biochar treatments had high HIX values compared to the other biochar feedstocks, while eucalyptus and filtercake biochar treatments were similar to the control (Figure 4.5C). Swine manure biochar at 400°C and filtercake at 400 and 500°C had low HIX values, consistent with their high FI and BIX values, meaning they contained less humified DOM from microbial rather than terrestrial sources. The HIX values for eucalyptus biochar treatments were around 10 and lower, suggesting that DOC leached from these treatments was slightly humified for the higher temperature biochars and less humified for the lower 400°C biochar.

4.4.4 PARAFAC analysis

The PARAFAC components for DOC identified in our study, based on Fellman et al. (2010), coincided with the fluorescence indices for the different treatments described above. Component 3 was greatest for cotton biochar treatments and swine manure biochar at 500 and 600°C. This is consistent with their low FI and BIX and high HIX values. Component 3 has been characterized as both oxidized quinone-like (Ishii and Boyer, 2012) and as reduced quinones that are more aromatic than oxidized quinones (Cory and McKnight, 2005). The Component 3 identified by Uchimiya et al. (2013) with 250/470, 350/470 peaks was also UVC humic-like and decreased with increasing temperature of pyrolysis, while Component 3 in our study appeared to either remain the same or increase with temperature (Table 4.3).

Filtercake biochars and swine manure biochar at 400°C had high Fmaxs for Component 2, consistent with their high FI and BIX and low HIX. The relative abundance of Component 2 lowered with increasing temperature of pyrolysis for all biochar feedstocks (Table 4.3). Other authors (Lin et al., 2012; Uchimiya et al., 2013) have likewise noted that contributions of Component 2 humic-like fraction decreased with increasing temperature as the humics fraction (humic and fulvic acid) was reduced. In addition, being UVA humic-like, Component 2 is susceptible to photodegradation from UVA light (Ishii and Boyer, 2012).

Eucalyptus biochar treatments and the control were dominated by Component 1, corresponding to their low FI and BIX, but not with their low HIX. Component 1 represents more oxidized fluorophores (Cory and McKnight, 2005), but here, the low HIX suggests the DOC was not humified. As DOC from eucalyptus biochar treatments was not much different from DOC from the control treatment, eucalyptus biochar probably did not contribute much to labile or leachable C, but may have prevented humified soil C from leaching.

Component 4 was the next most highly represented component for cotton biochars and swine manure 500 and 600°C biochars. This component increased with rising temperature of pyrolysis for most biochars, consistent with Uchimiya et al. (2013) who suggested the increase was due to the higher low-molecular weight acids fraction (Lin et al. 2012).

Component 5 was the only protein-like component, and it was high for swine manure 400°C and the filtercake biochars. Tye and Lapworth (2016) also identified a tyrosine-like protein component from soil DOM which was suggested to represent a more labile DOM fraction of microbial or plant cell sources. This is again consistent with swine manure 400°C and the filtercake biochar treatments’ high FI and BIX values and low HIX values. Swine manure and filtercake biochars themselves also had high total N content (Table 4.1), indicating they could contribute to proteins in the labile DOC. In contrast to the swine manure biochar in our study, Uchimiya et al. (2013) observed that poultry manure biochar had very little contribution from protein-like Component 5. However, the authors observed a decrease in Component 5 with increasing temperature of pyrolysis similar to that observed in our study. Component 5 was higher for biochars at 400°C compared to at 600°C for all biochar treatments in our study, except for filtercake biochar where it was highest at 600°C (Table 4.3). Component 5 may be related to lignin content, with biochar feedstocks with higher lignin content having a higher Component 5 contribution (Uchimiya et al. 2013). This would not explain the high Component 5 contribution in the swine manure 400°C biochar which would be expected to have a low lignin content, but it may explain the high Component 5 for filtercake 600°C. Similarly, another study with sugarcane filtercake biochar showed that the biochar retained more high-molecular weight, humic DOC species in the soil while the labile components were leached (Eykelbosh et al., 2015). The authors suggested that the filtercake biochar may have assisted in retaining humified components already existent in the soil.

4.4.5 Principle component analysis of DOC quantity and quality, as well as NO_3^- concentrations

PCA of DOC and NO_3^- concentrations and DOC characteristics from the different biochar treatments supported the results discussed above. Cotton biochar at 400°C was clustered near Group 1 (FI, BIX, Fmax5, and Fmax2) whose values were negatively correlated, indicating that DOC from the lower temperature cotton biochar was more labile and of microbial sources compared to the higher temperature biochars. In contrast, cotton biochars at 500°C and 600°C clustered closer to Groups 2 and 3 whose variables were positively correlated, suggesting DOC and NO_3^- concentrations in leachate increased with increasing soil C/N ratio and UVC humic-like and humic-like components (Fmaxs 1, 3 and 4) (Figure 4.7A). For swine manure biochars, the 400°C biochar was similarly clustered near the negatively correlated Group 2 (FI and BIX), reinforcing its primarily microbial sourced DOC. Swine manure at 500°C was near the negatively correlated Fmax4 and Fmax3 indicating its DOC was more humic-like, while swine manure at 600°C was clustered near C/N ratio, HIX, Fmax1, Fmax 2, and Fmax 5 which were positively correlated (Figure 4.7B). This suggests that the DOC from the higher temperature swine manure biochar was mostly humic with some protein and of terrestrial sources perhaps due to a higher lignin content compared to the lower temperature biochars.

Eucalyptus biochars at 400°C and 500°C were closer to Group 1, with HIX, DOC, and NO_3^- concentrations negatively correlated meaning that DOC and NO_3^- concentrations in eucalyptus biochar leachates decreased with less humified DOC. Thus DOC leached was mostly of microbial source rather than terrestrial, implying soil DOC was retained. Eucalyptus biochar at 600°C was closer to Group 2 whose variables were all positively correlated, suggesting that DOC sources from this biochar were a mix of microbial and terrestrial (Figure 4.7C). Lastly, filtercake biochar at 400°C was clustered by Group 2 whose variables were also positively correlated, except for the HIX which was negatively correlated. As with the other 400°C biochars (except for eucalyptus), the positive correlation between FI and BIX suggest an increase in more labile DOC with more biological, autochthonous DOC which would be related to less humified DOC (lower HIX ratios). Tye and Lapworth (2016) also observed a positive correlation between FI and β/α ratios with a strong negative correlation with HIX. The negative correlation with C/N ratio reemphasizes that DOC from filtercake biochar at 400°C was more labile since, as previously mentioned, higher lignin biochars may contribute to more protein-like (Component 5) than humic-like DOC (Uchimiya et al., 2013). Filtercake biochars at 500°C and 600°C were grouped by Fmax3 and DOC concentrations, suggesting that DOC concentrations from these biochars increased with terrestrially sourced DOC more than microbial sourced (Figure 4.7D).

4.5 Conclusions

The biochars used in this study contributed to both differences in DOC and NO_3^- concentrations in soil leachate and differences in DOC quality. Observing DOC concentrations leached from each treatment and the quality of the DOC, it is clear that certain feedstocks contributed to either the loss of fresher DOC or more humified DOC. As hypothesized, eucalyptus biochar treatments had very low DOC losses similar to the control, as did filtercake biochar treatments, and what DOC was leached from the soil was primarily more labile, microbial-derived DOC rather than terrestrial. Both feedstocks may have contributed to stabilizing more humified C components in the soil compared to the other two feedstocks. Treatments with swine manure biochar (especially at 400°C) lost higher amounts of DOC than the control, mostly from microbial sources, but also humified and terrestrial at higher temperatures. Similarly, treatments with cotton biochar lost the highest amounts of DOC mostly of humified and terrestrial origin. This, along with the high NO_3^- levels in its leachate, give reason to believe that cotton biochar would not help prevent nutrient leaching or stabilize C pools in an Arenosol, at least not until 6 weeks after application.

Overall, this study emphasizes how DOC and NO_3^- concentrations in leachate can vary considerably depending on biochar feedstock, and that DOC quality can be affected by both the feedstock and the temperature of pyrolysis. Fluorescence spectroscopy with EEMs PARAFAC analysis provided useful information on DOC quality. This information can assist in determining the right feedstock and temperature of pyrolysis to produce a biochar suitable for the producer’s needs (e.g. high FI and BIX, low HIX, such as for filtercake biochars). Of the four biochar feedstocks, filtercake and eucalyptus show the most promise for retaining DOC and NO_3^- in a Brazilian Cerrado Arenosol, but combinations of biochars (e.g. cotton with filtercake or swine manure with eucalyptus) may produce additional benefits and remain to be tested.

Chapter 5: Conclusions

5.1 Summary of experimental results

This thesis aimed to evaluate the potential of biochars made from four local agricultural wastes to improve Cerrado Arenosol soil properties and maize growth, as addressed by the research questions in **Section 1.6**. To our knowledge, this is the first study examining the use of biochar as a soil management practice for maize production on Cerrado Arenosols.

In **Chapter 2**, the contribution of these four biochar feedstocks pyrolyzed at 400°C, 500°C, and 600°C to soil water retention in Arenosols under maize was tested at a 5% w/w application rate, as well as examining the physical properties of the biochars that can affect soil water retention. Soils with filtercake and eucalyptus biochars contributed to high maize biomass, in particular filtercake biochar at 600°C which increased maize biomass 33% more than the control. Cotton and swine manure biochars, in contrast, contributed to the lowest maize biomass. These differences are likely related to the biochars particle size and contribution to grain size distribution when mixed in the soil. The larger coarse sand fraction in soils with filtercake and eucalyptus biochars compared to soils with cotton and swine manure biochars, which had higher fine silt+clay fractions, likely led to improved aeration and water movement rather than to excessive water retention and EC. Nevertheless, all the biochars showed potential to reduce water drainage as well as increase mean plant AWC in the soil compared to the control (no biochar), suggesting that at a lower application rate (<5%), cotton and swine manure biochars may still contribute to soil water retention without negatively impacting plant growth. In particular, this study highlighted possibly the first environmentally beneficial use of the waste biomass, filtercake.

Chapter 3 considered the effects of cotton, swine manure, eucalyptus, and filtercake biochars, each produced at 400°C, on maize biomass when added at increasing application rates (1-4% w/w). Filtercake biochar at almost all application rates led to the highest mean biomass compared to the other biochars and the control, followed by biomass in soils with eucalyptus biochar. Eucalyptus biochar, however, did not contribute much to soil fertility besides increasing soil total C, while filtercake biochar led to high soil nutrients, e.g. Ca, Fe, Mn. Soils with cotton and swine manure biochars produced the lowest maize biomass, decreasing with increasing application rate, but swine manure biochar was rich in N and other nutrients, as is typical of biochars made of animal waste. The low plant biomass observed in cotton and swine manure biochar treatments was likely due to higher pH, salinity, and/or excessive water retention. High salinity in particular may have affected the plants' ability to uptake water and nutrients, affecting their growth. This study emphasized the importance of testing and selecting the right application rate in a controlled setting before adding biochar to the field, as well as determining which biochar type to use based on the soil or crop needs.

In **Chapter 4** the effect of the twelve biochars on DOC and NO_3^- in soil leachate was examined. DOC concentrations, as well as NO_3^-, were low in leachate from soils with filtercake and eucalyptus biochars, and high in leachate from soils with cotton and swine manure biochars. The high application rate (5% w/w) used in this study likely contributed to the high NO_3^- leaching from the latter biochars, as they show potential to increase soil total N levels, but do not appear to retain it well in the soil. Analysis of DOC using fluorescence spectroscopy and PARAFAC analysis showed that DOC quality varied significantly by both biochar feedstock and temperature of pyrolysis. Swine manure at 400°C, filtercake, and (to a lesser extent) eucalyptus biochars mostly led to DOC losses of microbial, labile sources, while cotton and swine manure biochars at higher temperatures led to DOC losses of mostly humified and terrestrial sources. At lower application rates (<5%) and at lower temperatures of pyrolysis (e.g. 400°C), combining the feedstocks (e.g. swine manure with eucalyptus or cotton with filtercake) may help prevent high DOC and NO_3^- losses while providing labile substrate for mineralization.

In summary, all four biochar feedstocks provided different levels of soil benefits. While there was no one biochar that both increased all available soil nutrients and soil water content the most, filtercake biochar provided the greatest soil benefits leading to higher mean maize biomass compared to the other biochars and even the control. Still, the other biochars each also provided certain benefits. Combining the different biochars could thus combine their individual properties to enhance several soil characteristics. These studies provide a closer inspection of the various contributions of four biochar feedstocks that are considered wastes, particularly cotton, swine manure, and filtercake. The results show the potential of an alternative use of these wastes as biochar, which could lead to environmental and economic benefits.

5.2 Alternative use of agricultural wastes

Charcoal in Brazil is mainly produced from wood, thus depending on tree plantations such as eucalyptus or native vegetation. Biochar, in contrast, can be made from any organic material, including biomass waste. Transforming animal and crop waste into biochar not only significantly reduces their weight and volume, but also provides possible economic opportunities by reducing energy use for recycling and waste reduction, reducing energy for long-distance transport for waste disposal, and recovering energy from waste. These benefits in turn indirectly contribute to climate change mitigation, reducing CH_4 emissions from landfills and increasing C sequestration in forests by conserving native vegetation (Lehmann and Joseph, 2015a). The potential for biochar transformation of the agricultural wastes used in this thesis are examined below.

5.2.1 Cotton residue

The state of Mato Grosso is the largest producer of cotton in Brazil, producing 1.3 million t and covering an area of about 448, 000 ha (IBGE, 2011). As such, it produces a large amount of post-harvest cotton residues. These residues are either left on the ground after harvest or removed and disposed of. The cotton husks (the shell that encloses the fibrous cotton flowers that are harvested) and cottonseeds can be transformed into byproducts such as oil and flour for human consumption or mixed into animal feed, as they are rich in oil and protein. However, despite its nutritional value, the use of cotton residue as a commercial byproduct is limited due to the fact that it contains gossypol, a natural toxic phenol. Cotton residue thus needs to be treated first to remove toxicity (de Araújo et al., 2003), increasing costs and discouraging some from using it.

Transforming cotton residue into biochar showed that it could contribute to C and water retention in the soil (**Chapters 2** and **3**). However, in order to be beneficial to plant growth, it needs to be applied at low quantities, e.g. 0.5-1% w/w, to prevent high salinity and/or excessive water retention. The high availability of cotton residues in Mato Grosso makes it a practical biochar feedstock to contribute to C build-up and WHC of Arenosols.

5.2.2 Swine manure

In Brazil, pig farming has grown considerably in the last few decades, as has the amount of waste the industry produces. Proper disposal of swine manure is important as it contains significant amounts of N and P, heavy metals, pathogens, hormones and antibiotics. Intensive pig-raising in confined animal feeding operations (CAFOs), the standard practice in Brazil, means a high number of animals are confined to a small space, increasing risks of environmental contamination (i.e. runoff into water bodies, leaching into groundwater, introduction of pathogens to the soil, and GHG emissions). Disposal of swine manure at present is typically through direct application to the soil surface, often without any treatment (Kunz et al., 2005). It is often applied at rates exceeding crop requirements, causing N and P leaching and direct and indirect N_2O emissions (Cowie et al., 2015).

Pyrolysis, which is usually above 350°C, eliminates pathogens (Lehmann and Joseph, 2009), making swine manure biochar safe to apply to the soil. In addition, swine manure biochar made at high temperatures of pyrolysis (e.g. 600°C) is less labile, reducing potential DOC and NO_3^- losses from the soil (**Chapter 4**). Pig raising accounts for 17% of the projects in Brazil approved by the Kyoto Protocol's Clean Development Mechanism (CDM), established for countries with emission-reducing commitments to invest in projects that reduce GHG emissions in developing countries (Sato and Azevedo, 2008; UNFCCC, 2014). Since raw manure releases the powerful GHGs N_2O and CH_4, pyrolyzing manure right away on location for storage and transport can reduce these emissions. Pyrolysis gases can be burnt for heat or electric energy, further avoiding GHG emissions (Cowie et al., 2015) and contributing to CDM goals. However, some level of GHGs will be emitted during handling, for example during the period that swine manure is dried to reduce the moisture content before pyrolyzing. In the case of the swine manure biochar used in this thesis, the raw manure was allowed to air-dry under the sun for two days before transporting to the commercial biochar producer. Moisture content was reduced to 45%, facilitating transformation into biochar (Álvaro Soares, SPPT Ltda., personal communication).

In our greenhouse study, swine manure biochar increased soil N more than the other biochar feedstocks used in this thesis, as well as contributed to soil water retention (**Chapter 2**) and soil nutrients (**Chapter 3**). The state of Mato Grosso has high swine production, with almost 2 million pigs sent to the slaughterhouse in 2010 (EMBRAPA, 2011). Although further research on the effect of swine manure on N_2O and CH_4 emissions, as well as N and P leaching and volatilization, is required, particularly at the field scale, swine manure shows great potential as a biochar feedstock. With high availability in Mato Grosso and applying at low rates to prevent excessive initial DOC and NO_3^- leaching (**Chapter 4**), swine manure biochar could improve soil properties for plant growth in Cerrado Arenosols while providing a safer, less polluting disposal alternative for the raw material.

5.2.3 Sugarcane filtercake

Filtercake is a dense, earth-textured material left over from filtration of sugarcane juice after clarification. It is often disposed of through direct field application, but because it leads to rapid mineralization, it poses risks for nutrient leaching and runoff if over-applied, causing eutrophication in bodies of water. In addition, its high water content complicates application and transportation, increasing costs for disposal (Eykelbosh et al., 2014). Sugarcane cultivation is tied to biofuel production in Brazil. Biomass electricity, mostly from combustion of sugarcane bagasse (the fibrous residue leftover after milling), accounts for 6.8% of Brazil’s domestic electricity production (EPE, 2013; Eykelbosh, 2014). While sugarcane bagasse is considered a valuable byproduct used as a biofuel to produce electricity for the distillery and electrical grid, sugarcane filtercake is considered a true waste product (Eykelbosh et al., 2014; George et al., 2010). Considering about 730 million t of sugarcane is produced in Brazil (IBGE, 2016), finding more efficient, low impact ways to dispose of the leftover filtercake is necessary. Transforming filtercake into a more stable product such as biochar could therefore give it a more environmentally beneficial purpose while simultaneously alleviating disposal problems.

This thesis showed that filtercake biochar increased several soil nutrients (e.g. Ca, Mg) and plant physiological properties (e.g. WUE_{prod}, NUE_{prod}), as well as increased mean maize growth compared to the control (particularly pyrolyzed at 600°C) and prevented DOC and NO_3^- leaching (**Chapters 2, 3 and 4**). It did not lead to significantly more water retention, but it provided higher mean AWC than the control and improved soil aggregation which could affect soil aeration and water flow (**Chapter 2**). Thus, filtercake biochar showed the greatest potential as a soil amendment to improve soil moisture dynamics, soil fertility and plant biomass compared to the other biochar feedstocks and could prove even more useful when combined with more C-retaining biochars such as cotton and eucalyptus biochars. Since 14.7 million t of sugarcane is produced in Mato Grosso on ~216,000 ha (IBGE, 2011), sugarcane filtercake could be an accessible feedstock for biochar in the region.

5.2.4 Eucalyptus residue

Like sugarcane bagasse, eucalyptus wood waste can be used as biofuel to produce energy for electricity, heat for drying commodity grains, or other purposes. It is particularly used in charcoal production for the metallurgy industry in Brazil, plantations of which are dedicated for this purpose (Petter et al., 2012). A metallurgical company with 30,000 ha of eucalyptus can produce 144,000 t of charcoal annually using hot-tail kilns. Eucalyptus plantations are harvested in 5-year rotations, yielding an average of 275 m^3 wood ha^{-1} (Bailis et al., 2013). Bailis et al. (2013) calculated this biomass C as representing 737 kg of sequestered CO_2. Thus burning the biomass releases a considerable amount of CO_2. As biomass use for energy is considered a

CDM, eucalyptus plantations are part of renewable resource projects in Brazil (Petter and Madari, 2012). Of the CDM projects approved in Brazil, 47% are for renewable resources (Sato and Azevedo, 2008).

Using plantations and wood waste can reduce use of native vegetation for charcoal production; transforming eucalyptus residue into biochar can provide an additional co-product and benefit. A waste product of sawmills and logging companies, eucalyptus residue can help reduce production costs compared to use of eucalyptus firewood. Moreover, diesel consumption can be reduced by minimizing transportation of firewood over long distances (Donizeti et al., 2006). Although eucalyptus biochar was not observed to contribute much to soil fertility or water retention (**Chapter 2**), it did not have detrimental effects on plant growth (**Chapter 2 and 3**), did not lead to high DOC and NO_3^- losses (**Chapter 4**), and showed potential for increasing C retention in the soil. Considering eucalyptus' importance in charcoal production for the metallurgic industry it is less likely to be diverted for biochar production compared to the other biomass residues used in this thesis. However, with the right incentives, charcoal producers could also produce eucalyptus biochar as another co-product with bio-oil and bio-gas, thus contributing to C sequestration.

5.3 Potential impacts of biochar on maize production in Brazil

Maize is the most extended cereal crop produced in Brazil, covering an area of ~15 million ha and producing ~70 million t of grain from both the main maize crop and the *safrinha* (dry season maize) in 2012 (IBGE, 2016). It has high production potential, with government and private sector field trials in Brazil showing productivity reaching 16,000 kg ha^{-1}. Yet the national production average is only 4417 kg ha^{-1} for the main crop and 4045 kg ha^{-1} for the *safrinha* (Cruz et al., 2010). While most of the soybean produced in Brazil is exported, maize production is mainly for internal use. Based on the 2006 agricultural census, 44% of the maize produced in Brazil is used by the producer (mostly for animal and human consumption) and 56% is sold (IBGE, 2011) for animal feed, the chemical industry, and consumers (Cruz et al., 2010).

Increasing global food demand requires the need for high-yielding technologies that have low environmental impact and can intensify production on existing croplands to prevent further land clearing (Tilman et al., 2011). In Brazil, based on a 2006 census, 89% of maize producers produced maize for their own consumption rather than for commercial use. However, these subsistence producers had lower productivity (2913 kg ha^{-1}) compared to producers that sold their grain (4324 kg ha^{-1}) (IBGE, 2011). Cruz et al. (2010) noted that the difference in productivity was due to inferior technology. Since the majority (85%) of maize producers have croplands between 0.5 and 100 ha compared to producers with $\geq$2500 ha (0.1%) (IBGE 2011), most of the subsistence production is from small-scale producers. As described earlier for improving charcoal technology, continued financial incentives along with knowledge transfer and training through Brazil's BNDE programs or as part of the Kyoto Protocol's CDM projects could provide support to small-scale producers in order to increase crop productivity without increasing negative agricultural impacts.

Field studies with maize have shown yield increases when adding or leaving crop residues or mulch on the soil surface combined with fertilizer applications (de Carvalho et al., 2012; Lal, 1995; Verhulst et al., 2011). Similarly, biochar application with fertilizer has also been observed to increase maize yield in the field. A wood biochar increased maize yield up to 140% after 4 years in a fertilized Colombian savanna Ferralsol (Major et al., 2010), while Acacia bark biochar+NPK increased maize yield in 3 months to the equivalent of ~14,000 kg ha^{-1} compared to NPK fertilizer applications alone (~8000 kg ha^{-1}) and the control (no NPK or biochar, ~5000 kg ha^{-1}) in a Sumatran tropical soil (Yamato et al., 2006). Willow wood waste biochar with fertilizer applications had the highest maize biomass and yield (~9000 kg ha^{-1}) in a 102-day field experiment on an Australian tropical Ferralsol compared to fertilizer alone (control), compost+fertilizer, biochar+compost+fertilizer, and a combined biochar-compost+fertilizer treatment (Agegnehu et al., 2016).

In addition to yield increases, Yamato et al. (2006) suggested that fertilizer application could be reduced when combined with biochar since in their experiment, only half of the standard fertilizer amount used in the region was applied. Increasing efficient N use through the development, improvement, and transfer of agronomic practices that retain N in the soil can reduce N fertilizer applications while maintaining crop yields (Tilman et al., 2011). The use of manure-based biochars in particular, which have higher N content than wood biochars, can reduce N fertilizer application rates. Lower N fertilizer application not only implies lower crop management costs, but indirectly leads to reduced GHG emissions from less N fertilizer produced, as N fertilizer manufacture requires high amounts of natural gas (Cowie et al., 2015).

The greenhouse studies conducted in this thesis showed the potential of different biochars in improving soil fertility and water retention for maize growth in a Cerrado Arenosol. The next step for the biochars used is to test their effect on soil properties and maize yield in the field in a long-term experiment. To reduce number of treatments, only two of the four biochars could be used: filtercake biochar, which had the highest NUE_{prod} and WUE_{prod}, and swine manure biochar which contributed to high total soil N and θ (**Chapters 2 and 3**), and both made of feedstocks that are produced at high rates in Mato Grosso. Small amounts of cotton biochar could also be mixed with both biochars to ensure greater water retention (**Chapter 2**). Based on the results of this thesis and other biochar studies in the literature, these biochars combined with inorganic fertilizer, have strong potential to improve soil properties and thus increase maize yield in the field, contributing to higher maize production per area in Cerrado Arenosols.

5.4 Future research directions

The work carried out in this thesis aimed to examine the effects of different biochars on soil chemical and physical properties. Through the experiments performed, the contributions of each biochar and their ideal application rates were determined to be able to make the next step to the field. Since biochar's ability to retain ions decreases as it "ages", i.e. oxidizes, in the soil environment (Spokas, 2013), a long-term field experiment with these biochars is required. Besides field-scale performance, other questions that arise from the results of this study and remain to be tested in the future are examined below.

5.4.1 How do these biochars affect soil microorganisms?

To round out the analysis of the effect of the biochars used in this study on the soil environment, their influence on soil microorganisms also needs to be examined. As reported in this thesis, biochar can alter soil physical and chemical properties, and these in turn will affect soil biological properties. The formation of new habitats and changes to the soil physico-chemical environment after biochar amendment can affect microbial activities, abundance, and community structure. Biochar pores, especially macropores, can provide protective habitats in which bacteria, fungi, and protozoa can hide from preying microarthropods (Gul et al., 2015). Biochar's high pH affects what microorganisms will settle on and around biochar particles, with fungi likely dominating as they are more tolerant to a range of pH compared to bacteria which prefer closer to neutral pH. As biochar will also affect the soil environment's pH, the composition of the soil microbial community may also change, possibly increasing the fungi:bacteria ratio (Thies et al., 2015).

Although biochar itself does not contribute to as much mineralizable C and nutrients as the bulk soil, biochar's porosity, surface area and particle size can increase movement of soil water and nutrients into biochar pores, encouraging microbial population growth (Gul et al., 2015). Increased C mineralization in the soil after biochar addition can be due to biochar consumption by microorganisms, a priming effect (an increase in existing SOC turnover (Kuzyakov, 2010)) on the SOM, or abiotic release of biochar C (Ameloot et al., 2013). Ameloot et al. (2013) observed a significant increase in microbial biomass C in a sandy loam with swine manure digestate biochar (pyrolyzed at 350°C) and willow wood biochar (350°C and 700°C) compared to the control (no biochar). Similarly, in their review of biochar effect on soil biology, Gul et al. (2015) observed across a number of studies a trend of increased microbial biomass after biochar application in a range of soil textures (e.g. clay loam, loamy sand, sandy loam). However, some studies did not observe an increase, so the authors caution against generalizing the effect. Moreover, if a biochar is mostly dominated by micro- and mesopores (<50µm), limiting microbial access to water and nutrients within them, there could occur a short-term drop in microbial biomass C. Gul et al. (2015) suggest mixing compost or manure with the biochar application in order to increase available nutrients for soil microorganisms.

As reported in this work and in others, the effect of biochar on the soil environment will vary with the feedstock type and temperature of pyrolysis, and the same applies for its effect on soil microorganisms. As with their influence on the soil physical and chemical properties, the effect of the different biochars used in this study on soil biological properties will likely vary considerably. Besides testing their effect alone, combining compost with the biochars could lead to other additional benefits. The benefits of adding compost to the soil are well-known, mainly in relation to increased SOC and available nutrients (Stevenson, 1994). Compost combined with biochar can lead to crop benefits and improved nutrient cycling, particularly increased N use efficiency. Biochar addition has also been shown to stimulate the composting process and improve the final quality (Steiner et al., 2015). Thus, further studies can be carried out with the biochars used in this thesis to develop the best biochar-crop management practices for Cerrado Arenosols. Combining compost with the C-rich, but nutrient-poor cotton and eucalyptus biochars (**Chapter 3**), for example, might provide more substrate for soil microorganisms, increasing nutrient mineralization, while improving soil physical properties.

5.4.2 How do these biochars affect GHG emissions from soil?

Biochar has been proposed as a “clean energy technology that reduces emissions as well as sequesters carbon” (p. 143, Lehmann, 2007). Based on the soil total C increase in soils with the biochars used in this study compared to the control (**Chapter 3**), it can be suggested that these biochars can reduce decomposition and thus CO_2 emissions from the soil. Although priming of labile C has been observed when first adding biochar, there have not been any studies so far that show increased respiration of stable C (Cowie et al., 2015). In a short-term incubation study, Eykelbosh et al. (2014) observed a priming effect in filtercake biochar-amended soils compared to the control (no biochar). The slight CO_2 flux increase was attributed to the biochar’s labile C components. Despite the small increase, CO_2 emissions from soils with filtercake biochar were significantly lower than those from soils with raw filtercake. Agegnehu et al. (2015) observed that CO_2 flux over the full growing season from the biochar+fertilizer treatment was lower than fluxes from the compost+fertilizer and biochar+compost+fertilizer treatments, but not less than the control (fertilizer only).

Although most studies of biochar effect on N_2O emissions have been conducted in the laboratory, a meta-analysis of literature on the subject has shown that biochar can reduce soil N_2O emissions by 50% (Van Zwieten et al., 2015). In the field, Agenhehu et al. (2015) measured lower N_2O fluxes from the biochar+fertilizer treatment than from the other organic amendment treatments and was lower than the control by the end of the field trial. In Brazil, 87% of the N_2O emitted into the atmosphere comes from the agricultural sector; hence, biochar's potential to prevent N_2O emissions from the soil is highly relevant (Petter and Madari, 2012). In contrast, biochar effect on CH_4 emissions is less clear (Van Zwieten et al., 2015). In their mesocosm study with and without swine manure additions, Troy et al. (2013) did not observe changes in CH_4 fluxes from soils when adding two biochars (derived from swine manure and pinewood). Yet, due to its N and nutrient content, of the biochars used in this thesis, swine manure biochar might contribute to more N_2O and CH_4 emissions than the other biochars. The contribution of each biochar type to these GHGs remains to be determined, first at a laboratory or mesocosm scale and later at the field scale.

5.4.3 What is the LCA of these biochars?

Studies on the effect of these biochars on GHG emissions could contribute to a larger examination of the life cycle assessment (LCA) of the biochars. LCA is a tool used "to systematically quantify the total environmental impacts of a product or process" (p.769, Cowie et al., 2015) from start to finish. The biochar life cycle includes obtaining biomass, producing the biochar, and applying it to the soil. These steps must include the GHGs emitted from fossil fuels during retrieval, transportation, and processing of the biomass; building and operating a biochar reactor; transportation and biochar application to the soil; and any indirect emissions, e.g. N fertilizer production. To determine the biochar's mitigation value, the biochar life cycle needs to be compared to the life cycle of conventional soil and biomass practices (Cowie et al., 2015). Bailis et al. (2013) performed an LCA on traditional hot-tail kilns and newer metal container kilns used to produce charcoal in a Brazilian metallurgical firm. The authors found that the metal container kilns had better environmental performance in terms of GHG emissions, water use, and energy-return-on-investment, among other indicators.

There is presently great variation and uncertainty in LCA studies on biochar due to differences in biochar types, pyrolysis reactors, assumed effects when added to the soil, and calculation methods (Cowie et al., 2015). Uncertainties about biochar properties, production, and byproducts will reduce as biochar production technology improves. What continues to be uncertain is the impact and duration of biochar effect on crop growth and GHG emissions when applied to the field. Because of all the variation, it is impossible to generalize results based on a few LCA studies (Rödger et al., 2016). Bailis et al. (2013) stated that there were several uncertainties in their LCA study, such as the use of emissions records from past studies rather than taking direct *in situ* measurements. However, their work could be useful to other charcoal-producing countries (e.g. in Sub-Saharan Africa) interested in improving charcoal technology. For this reason, an LCA study on the biochars used in this thesis could serve to further knowledge on the environmental and socio-economic benefits of converting the feedstocks available in the Cerrado region into biochar.

5.5 Overall significance

The overall objective of this thesis was to evaluate four biomass wastes as biochar feedstocks to improve physical and chemical properties of a Cerrado Arenosol. The biomass wastes were chosen based on their prevalence in the state of Mato Grosso and their environmental impact.

This thesis provided an agronomic outlook of the potential of these biochars to improve soil conditions for crop growth, finding that filtercake biochar had the highest potential for increasing crop production. Further analysis of these biochars, such as their effect on soil biological properties, are required to have a more holistic image of their contribution to soil conditions. However, the soil and plant analyses carried out in this thesis provide the initial base for other research to build on. In addition, although this thesis does not include a social or economic study of the biochars used, it opens up discussion on more efficient waste disposal of the raw feedstocks in the form of biochar that could also create byproducts (i.e., bio-oil, bio-gas) to sell or reduce farm costs. The large charcoal industry in Brazil is already moving forward in improving pyrolysis technology for the metallurgic industry. The findings of this thesis, in particular the benefits of transforming sugarcane filtercake into a biochar soil amendment, can help convince metallurgic companies to invest in biochar production and benefit from CDM or BNDES funding, thus contributing to C sequestration and GHG mitigation.

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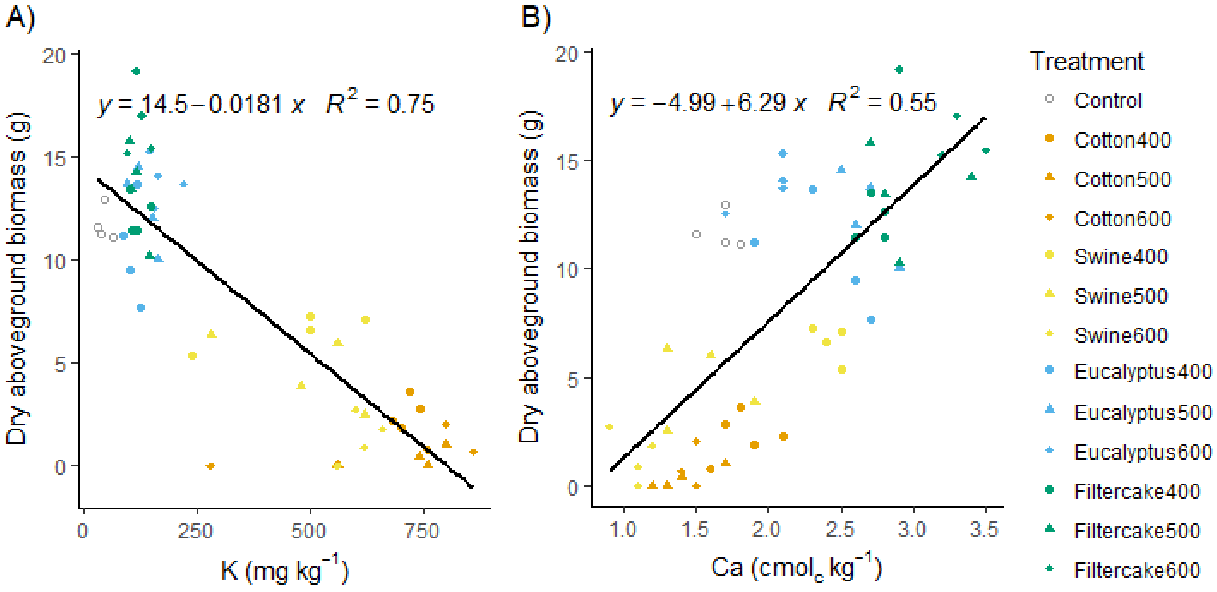
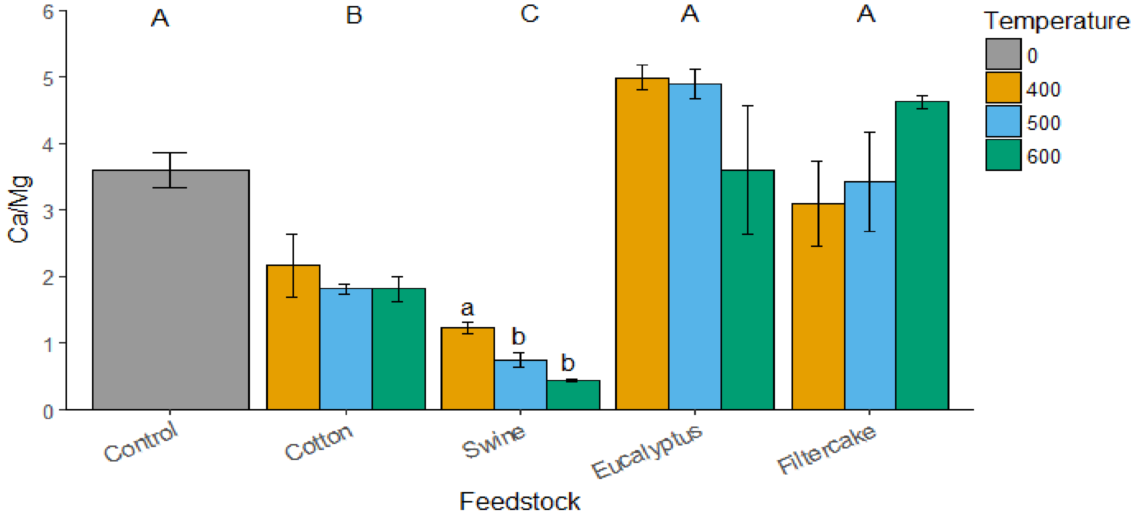
Appendices

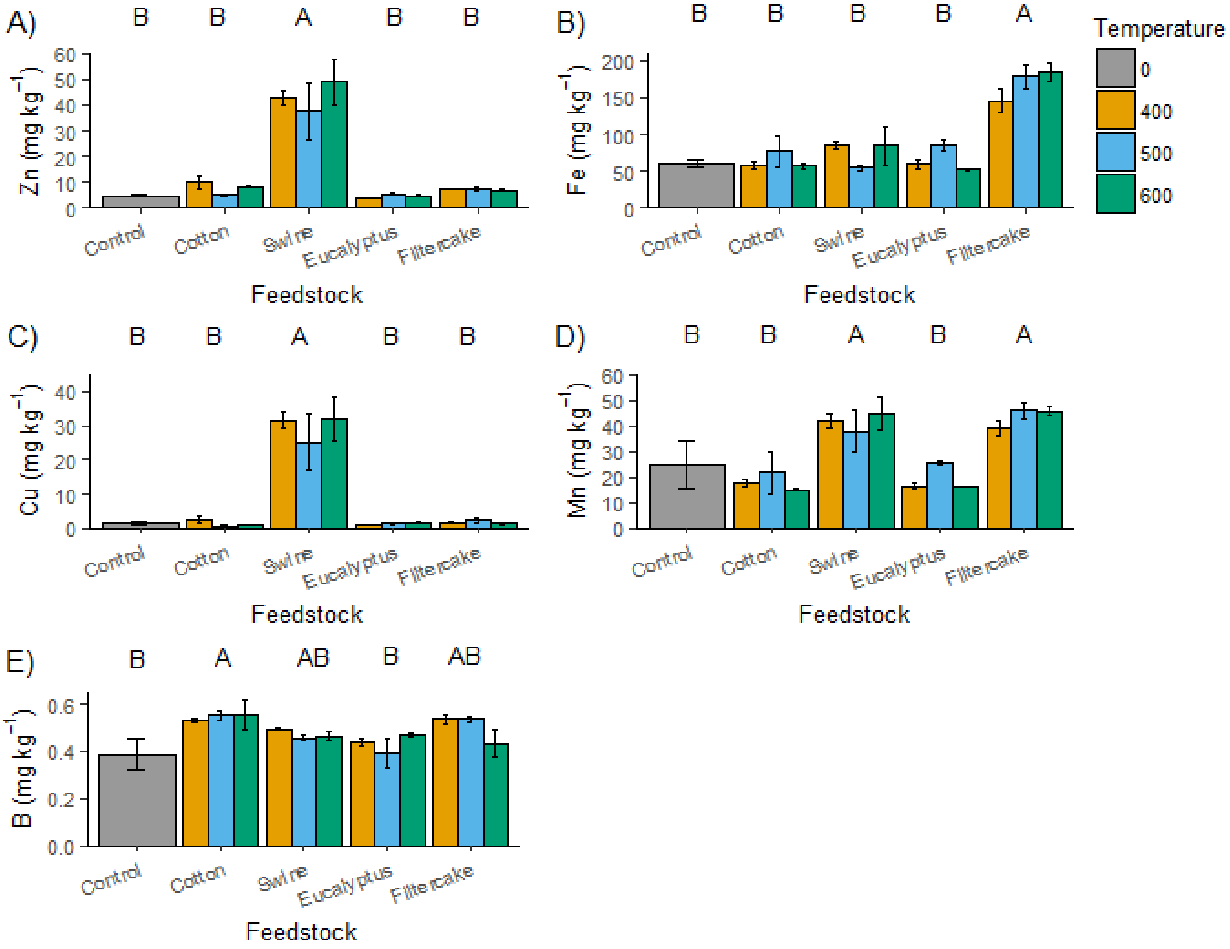
Appendix A Correlations between maize biomass and soil K and Ca concentrations, Ca/Mg ratios, and micronutrient concentrations in soils with 12 different biochars



Appendix Figure A.1. Correlations between dry aboveground biomass (g) and A) potassium (K; mg kg^{-1}) and B) calcium (Ca; cmol$_c$ kg^{-1})

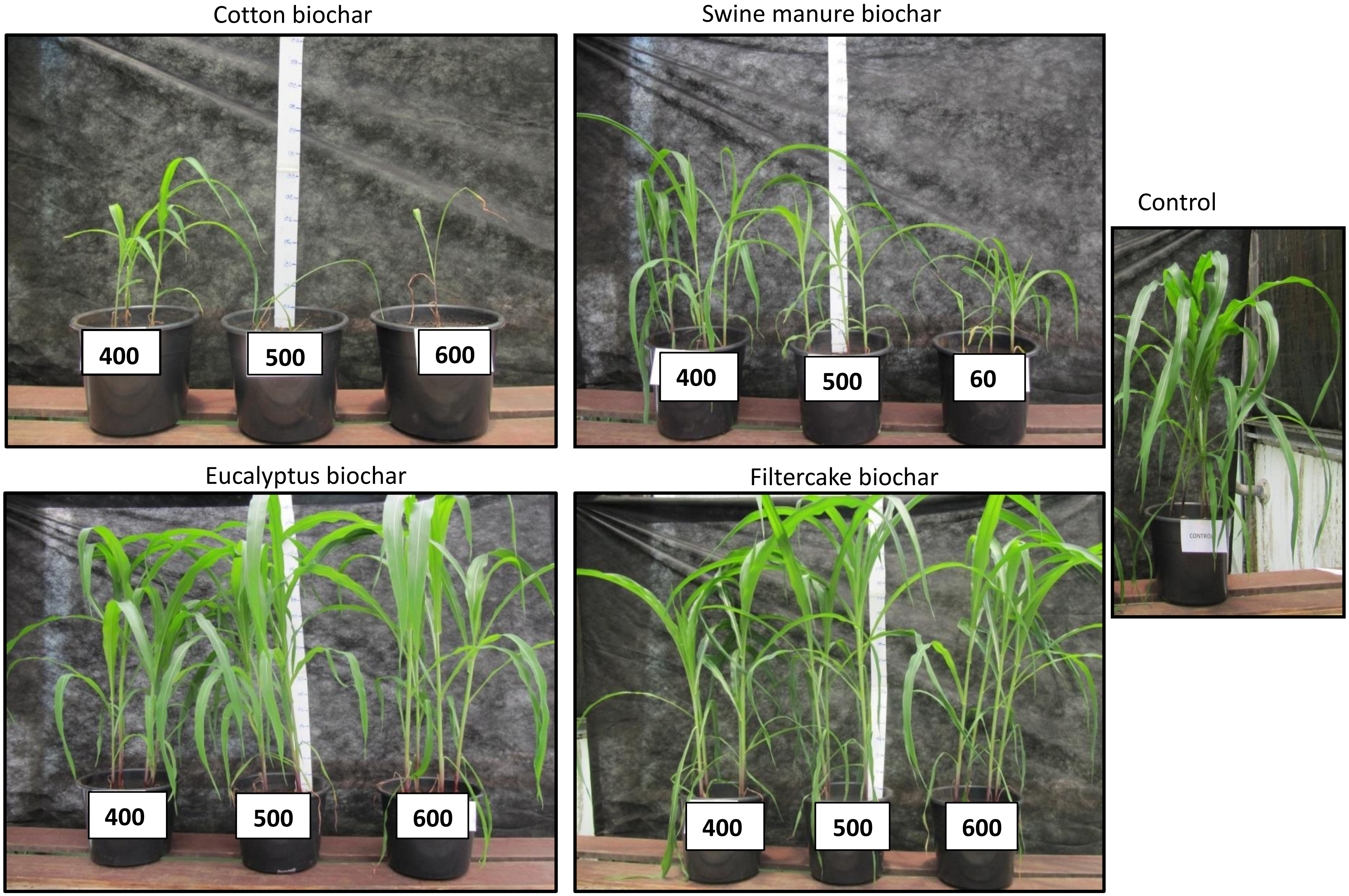


Appendix Figure A.2. Mean calcium/magnesium (Ca/Mg) ratios (*n* = 4). Capital letters represent significant differences between the feedstocks while lowercase letters represent significant differences between the temperatures (Tukey test; *P* < 0.05)



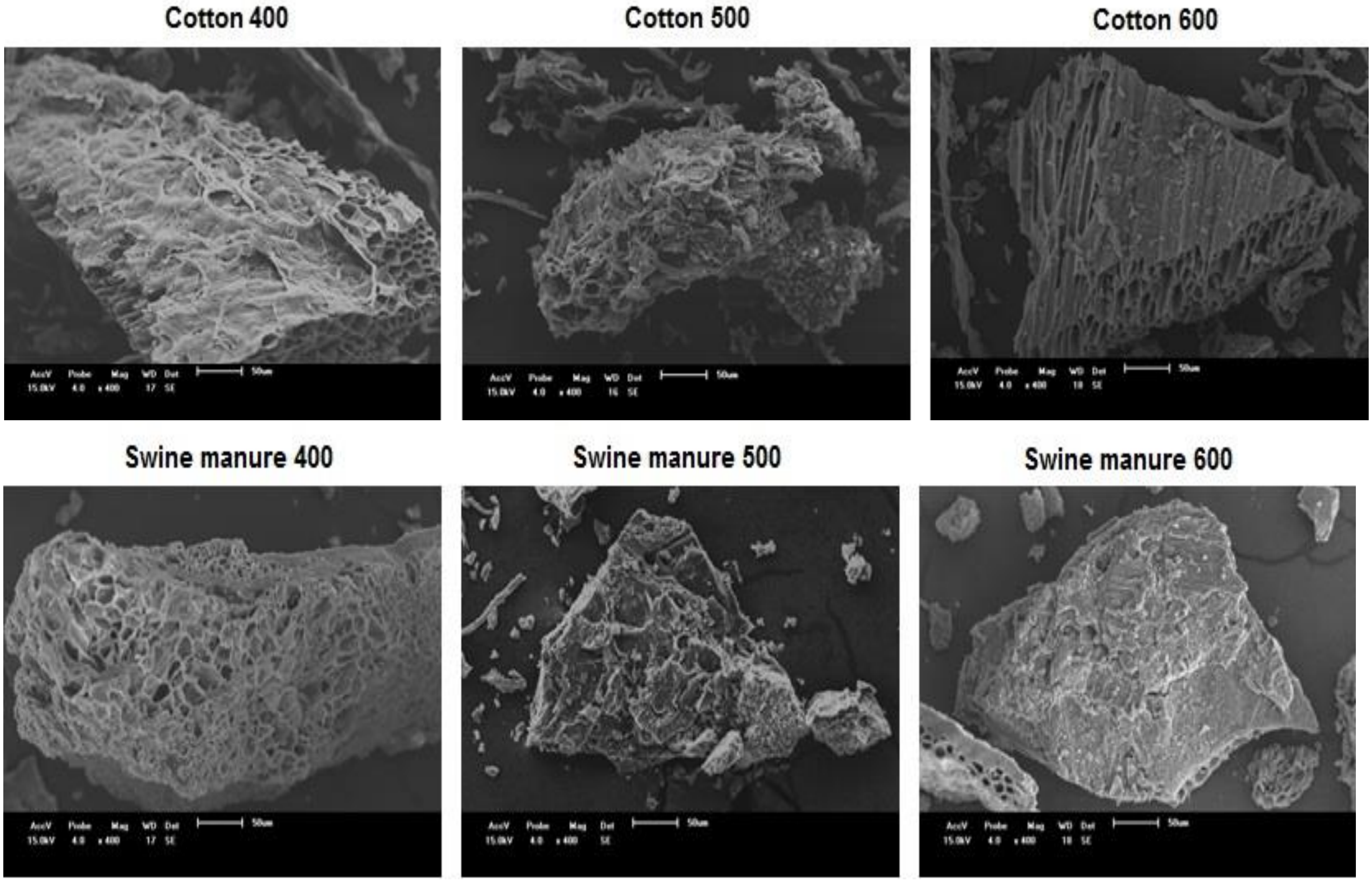
Appendix Figure A.3. Mean micronutrients A) zinc (Zn; mg kg^{-1}), B) iron (Fe; mg kg^{-1}), C) copper (Cu; mg kg^{-1}), D) manganese (Mn; mg kg^{-1}), and E) boron (B; mg kg^{-1}). Capital letters represent significant differences between the feedstocks (Tukey test; *P* < 0.05). There were significant differences between the temperatures for each feedstock.

Appendix B Maize biomass in soils with different biochar feedstocks and temperatures of pyrolysis

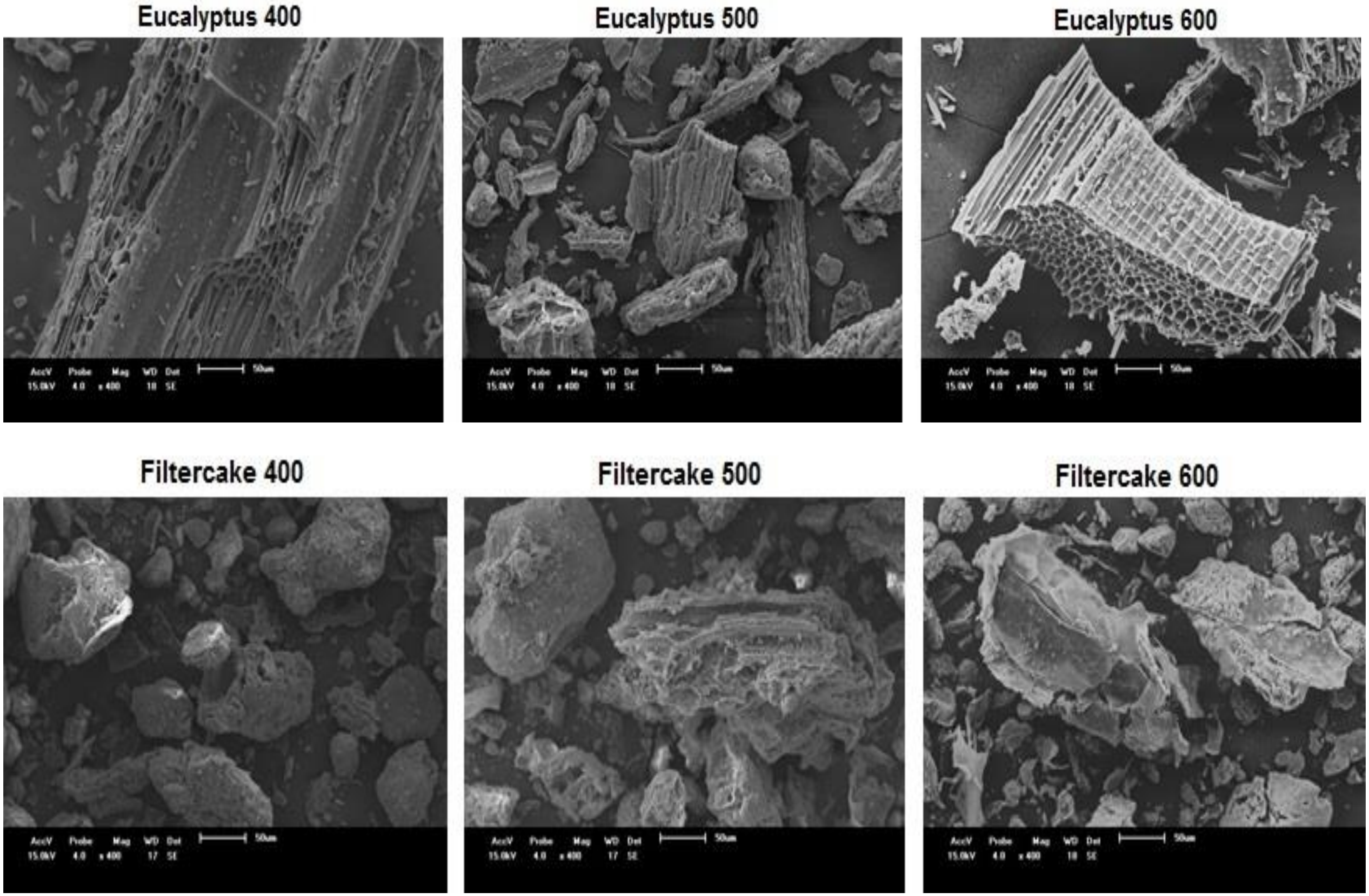


Appendix Figure B.1. Maize plants in soils with 12 different biochars: cotton, swine manure, eucalyptus, and filtercake biochars at 400, 500, and 600°C. Control treatment did not contain biochar.

Appendix C SEM images of different biochars



Appendix Figure C.1. SEM images of cotton and swine biochars at 400, 500, and 600°C at x400 magnification (Shimadzu SSX-550 Superscan microscope).



Eucalyptus 400

Eucalyptus 500

Eucalyptus 600

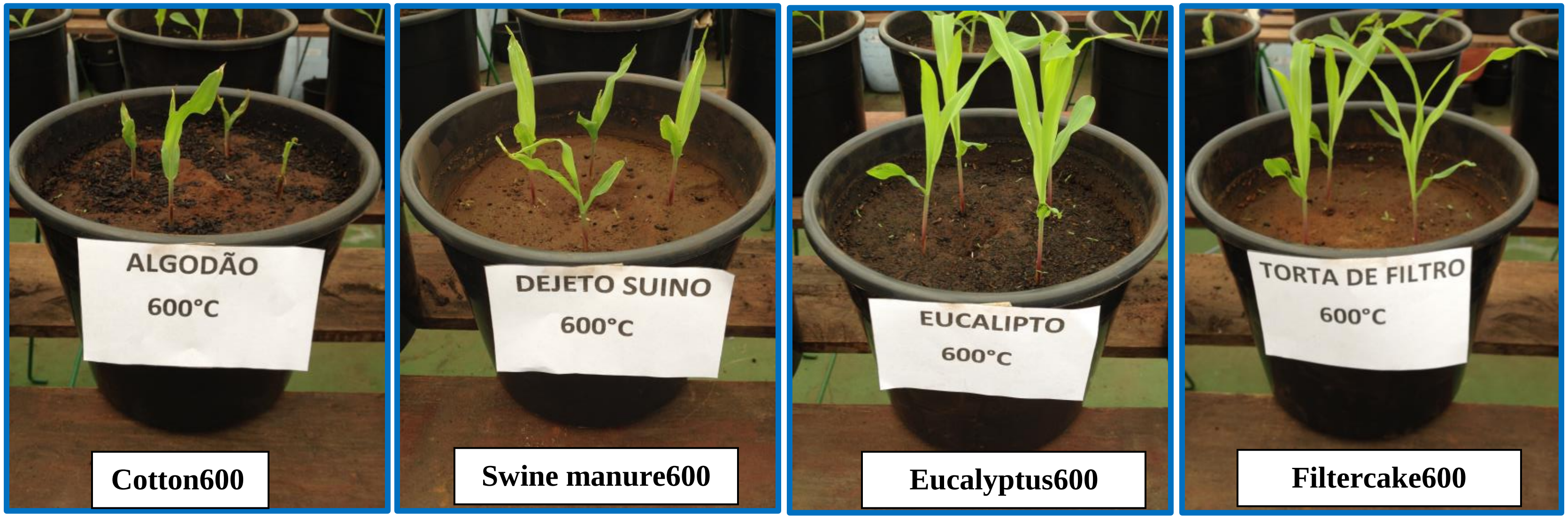
Filtercake 400

Filtercake 500

Filtercake 600

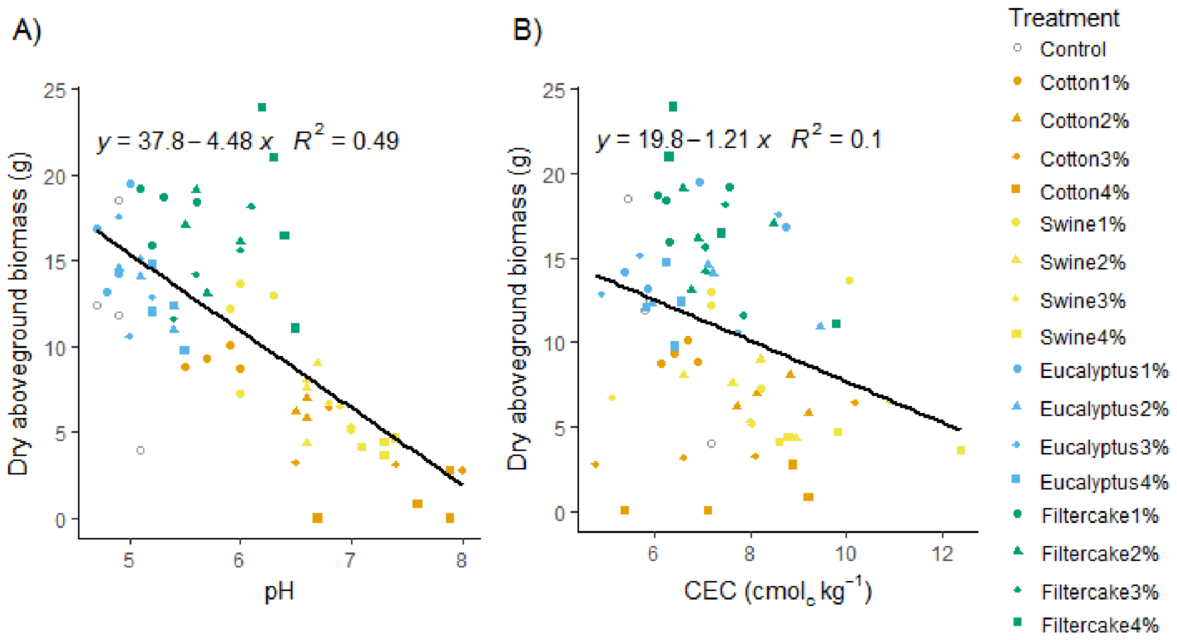
Appendix Figure C.2. SEM images of eucalyptus and filtercake biochars at 400, 500, and 600°C at x400 magnification (Shimadzu SSX-550 Superscan microscope).

Appendix D Maize biomass in soils with different biochar feedstocks pyrolyzed at 600°C temperature

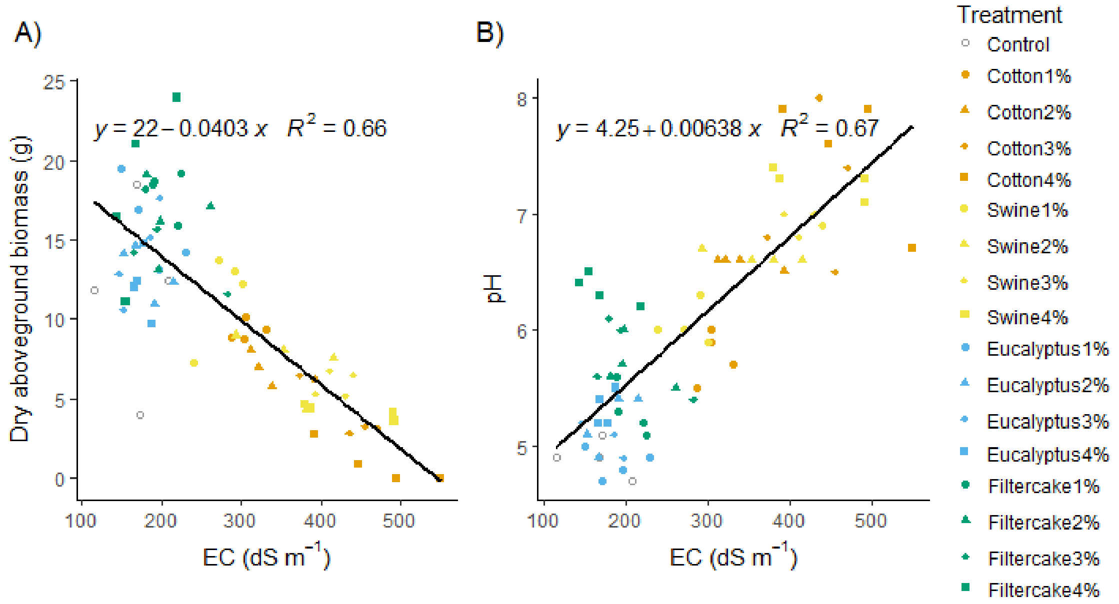


Appendix Figure D.1. Maize plants in soils with cotton, swine manure, eucalyptus, and filtercake biochars at 600°C in the second week of the experiment. Biochar is visible on the soil surface for some feedstocks more than for others.

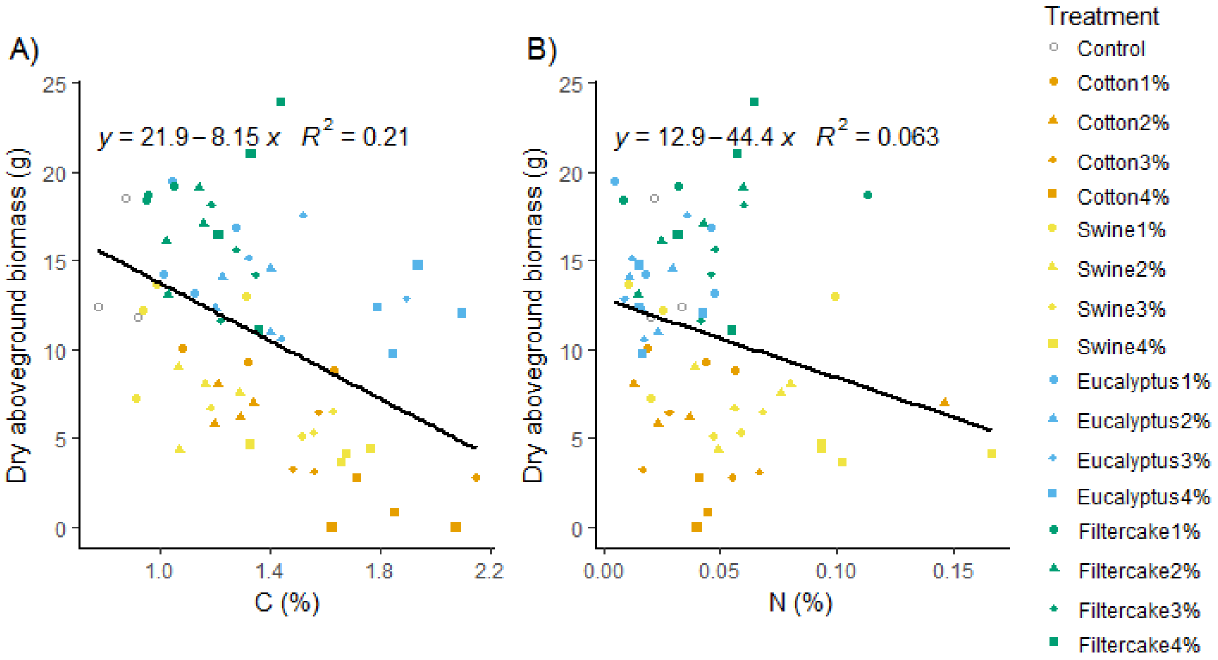
Appendix E Significant correlations between dry aboveground maize biomass and plant photosynthesis (P_{plant}) with soil properties in soils with different biochars at 1-4% application rates



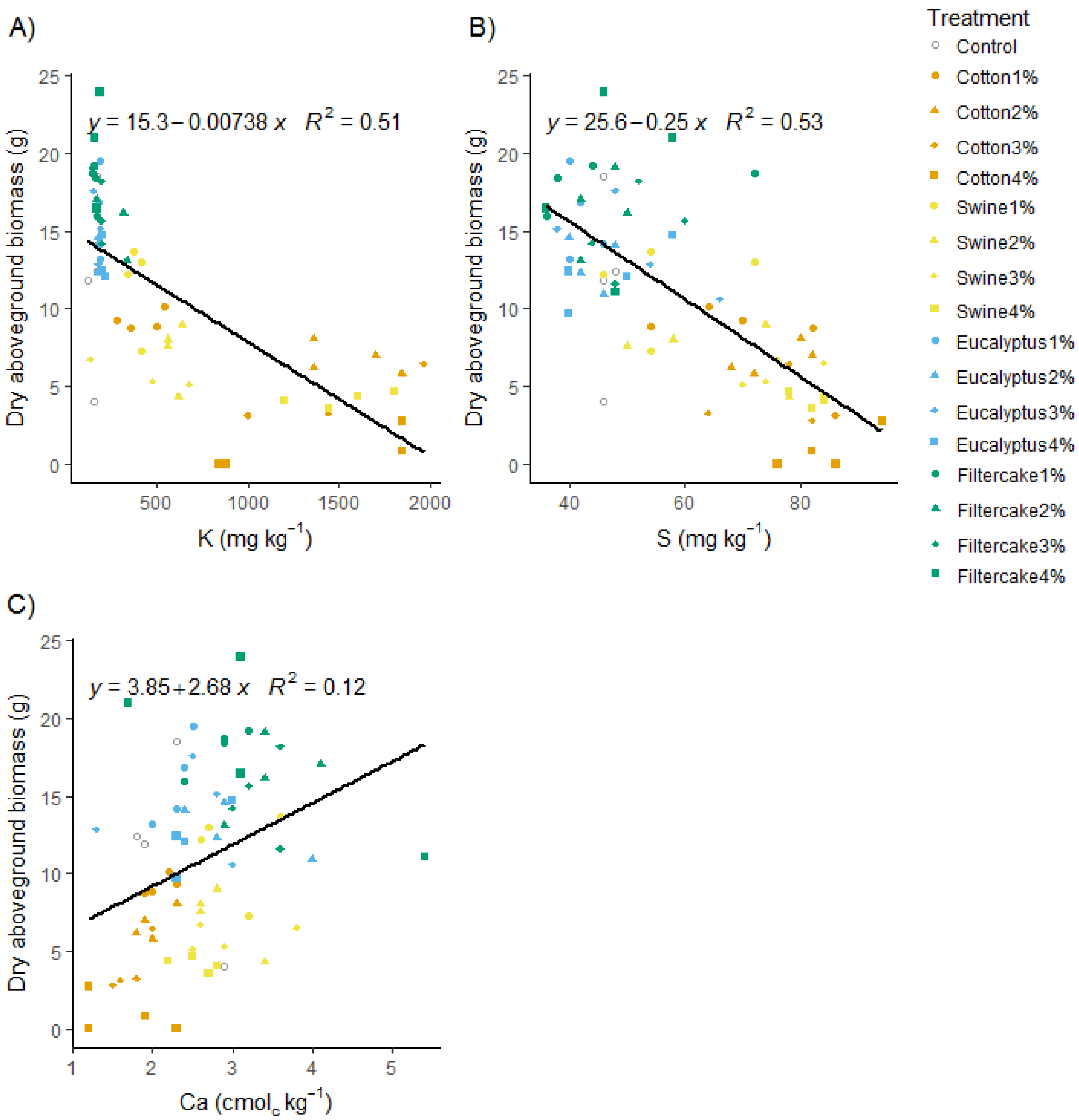
Appendix Figure E.1. Correlation between dry aboveground biomass (g) and A) pH and B) cation exchange capacity (CEC; $cmol_c$ kg^{-1}).



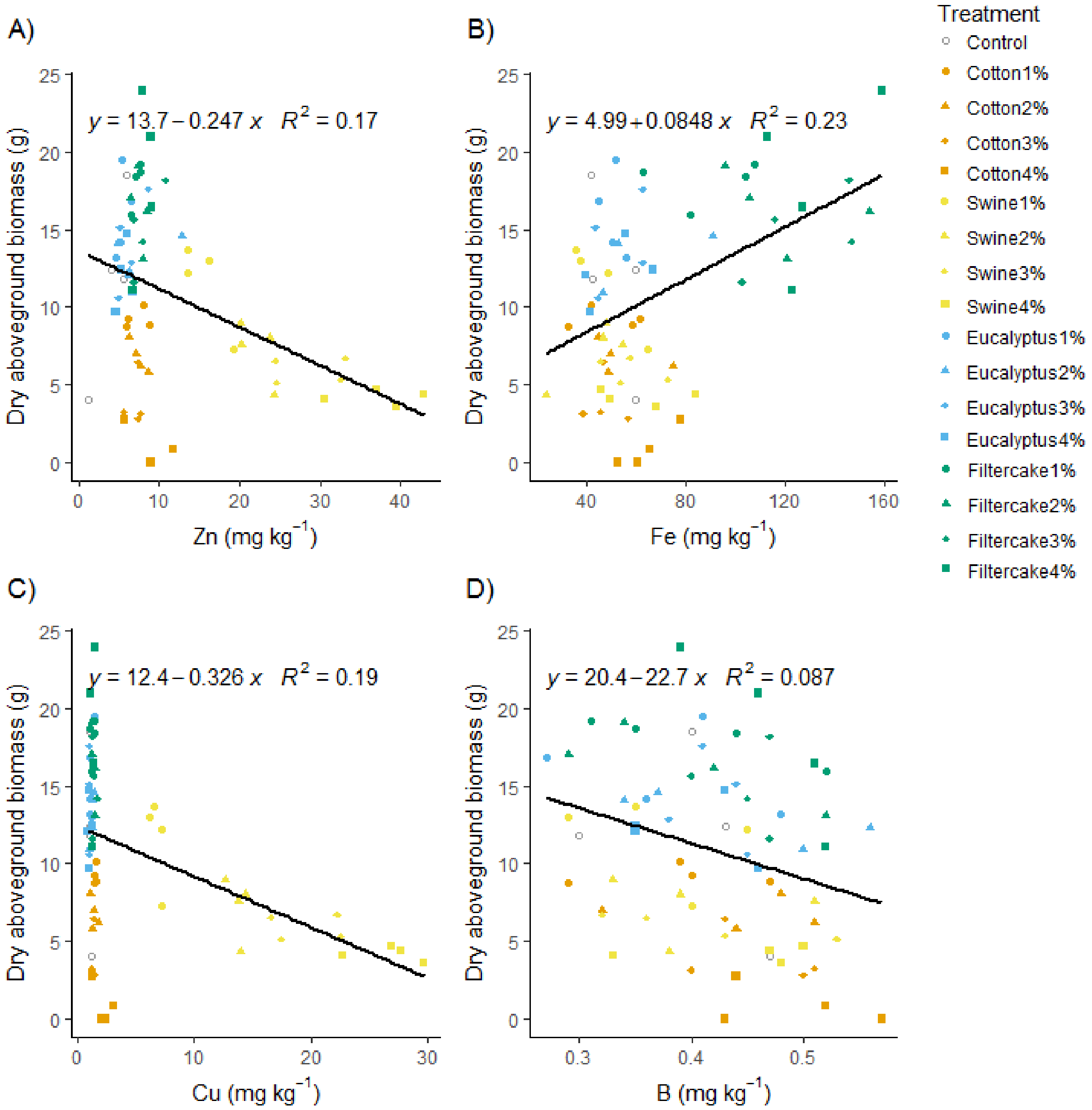
Appendix Figure E.2. Correlations between EC (dS m^{-1}) and A) dry aboveground biomass (g) and B) pH.



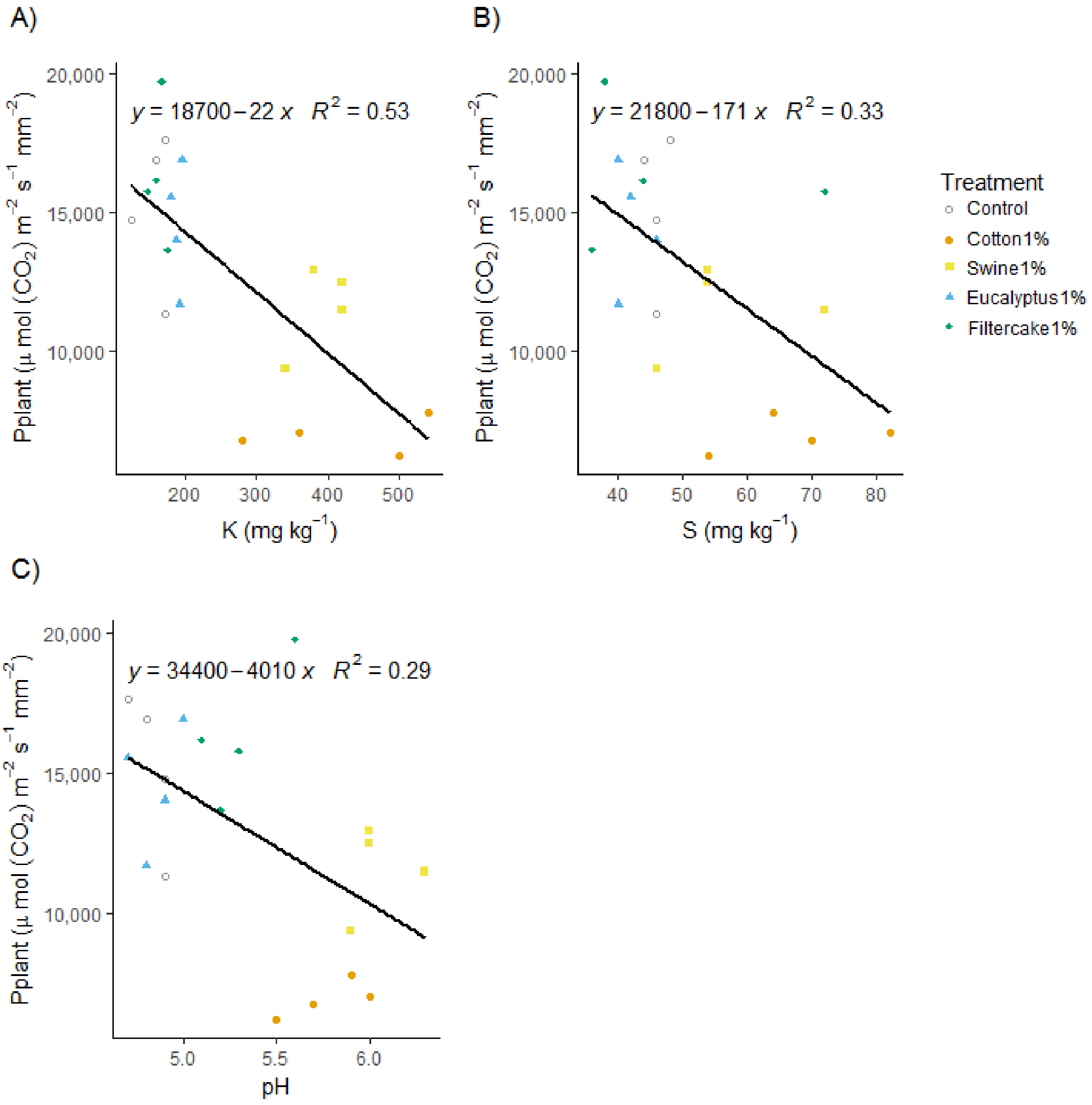
Appendix Figure E.3. Correlation between dry aboveground biomass (g) and A) C (%) and B) N (%).



Appendix Figure E.4. Correlation between dry aboveground biomass (g) and A) potassium (K; mg kg^{-1}), B) sulfur (S; mg kg^{-1}), and calcium (Ca; cmol$_c$ kg^{-1}).



Appendix Figure E.5. **Correlation between dry aboveground biomass (g) and A) zinc (Zn; mg kg^{-1}), B) iron (Fe; mg kg^{-1}), C) copper (Cu; mg kg^{-1}) and D) boron (B; mg kg^{-1}).**



Appendix Figure E.6. Correlation between plant photosynthesis (P_{plant}, μmol (CO_2)·m^{-2}·s^{-1}·mm^{-2}) and A) potassium (K; mg kg^{-1}), B) sulfur (S; mg kg^{-1}), and C) pH.

Appendix F Photographs of maize plants in a greenhouse experiment

F.1 Maize growth under different biochars at 1-4% application rates

Appendix Figure F.1. Maize plants in soils under control (no biochar) and cotton biochar treatments (doses 1-4%).

Appendix Figure F.2. Maize plants in soils under control (no biochar) and swine manure biochar treatments (doses 1-4%).

Appendix Figure F.3. Maize plants in soils under control (no biochar) and filtercake biochar treatments (doses 1-4%)

Appendix Figure F.4. Maize plants in soils under control (no biochar) and eucalyptus biochar treatments (doses 1-4%)

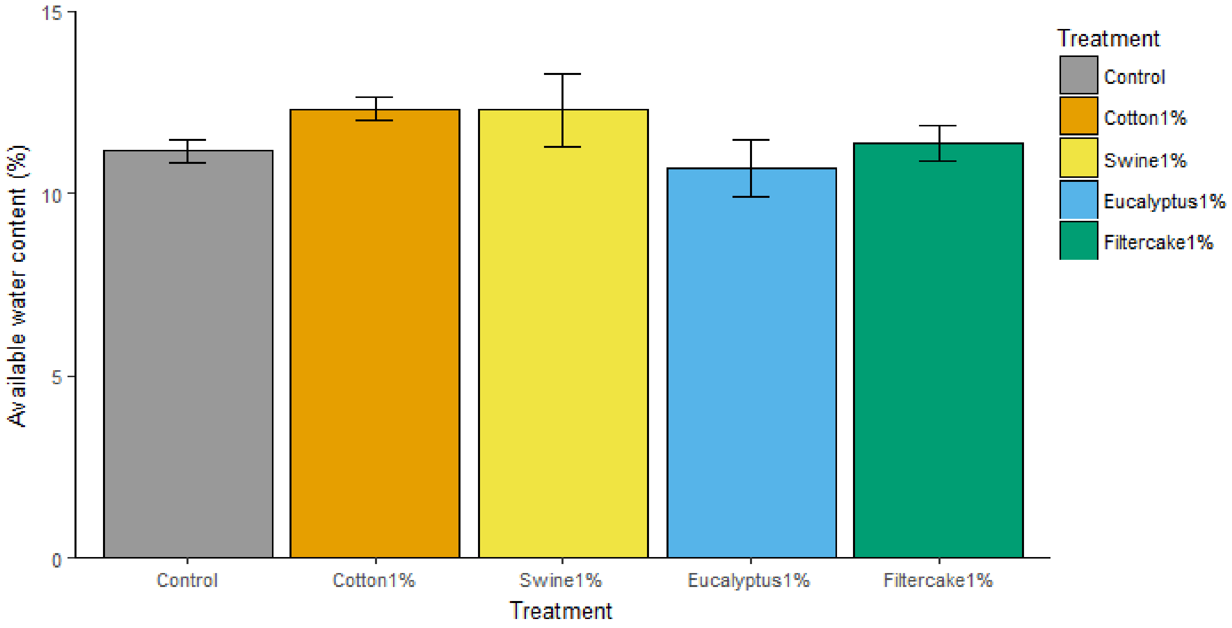
F.2 Signs of toxicity or deficiency in maize plants under different biochars and application rates

Appendix Figure F.5. Maize plants in soil with swine manure biochar at 1% (right) and 4% (left) doses. Plant leaves in the 1% dose showed slight crinkling at the edges, whereas at the higher 4% dose, leaves were not able to fully expand.

Appendix Figure F.6. Maize plants in soil with cotton biochar at 1% (right) and 4% (left) doses. Plant leaves show crinkling or ripping at the edges and browning/yellowing, especially at the higher 4% application rate.

Appendix G Plant available water content

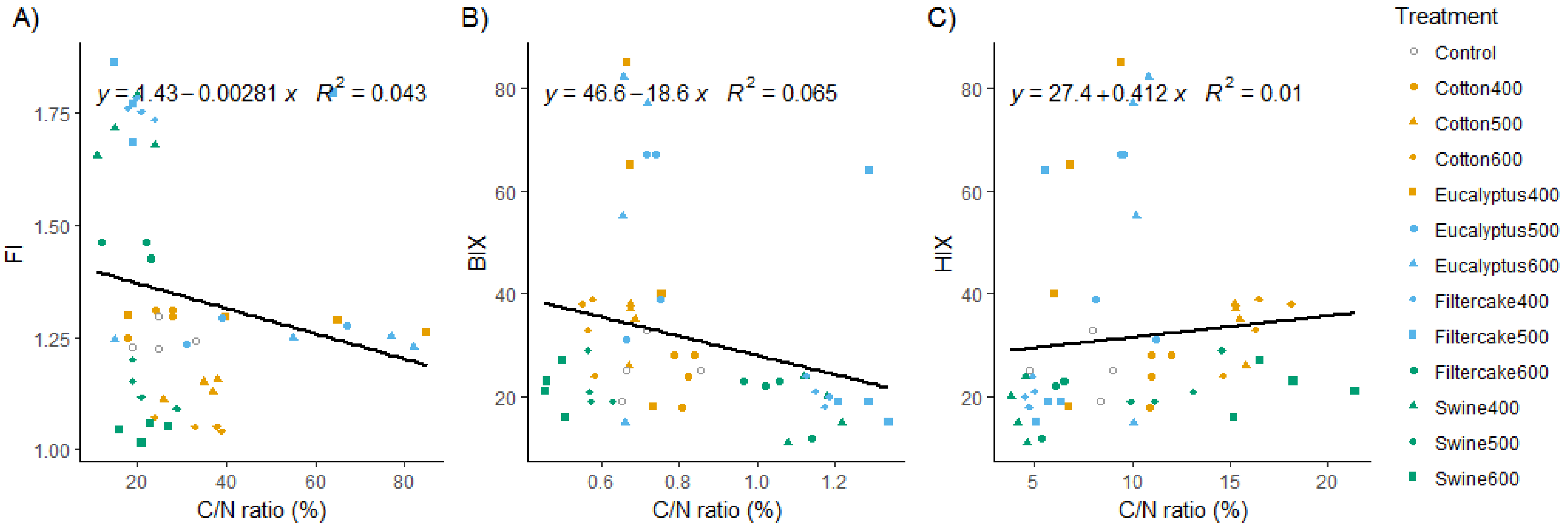
G.1 Plant available water content in soils with 1% biochar dose



Appendix Figure G.1. Available water content (%) in biochar treatments with 1% dose. There were no significant differences between the treatments.

Appendix H Analysis of DOC characteristics

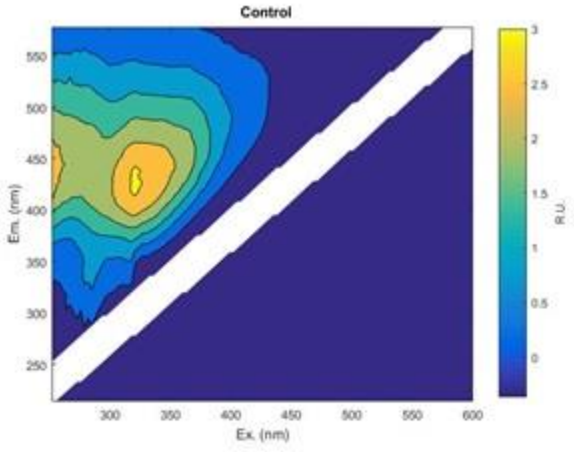
H.1 Relationship between DOC characteristics and soil C/N ratio



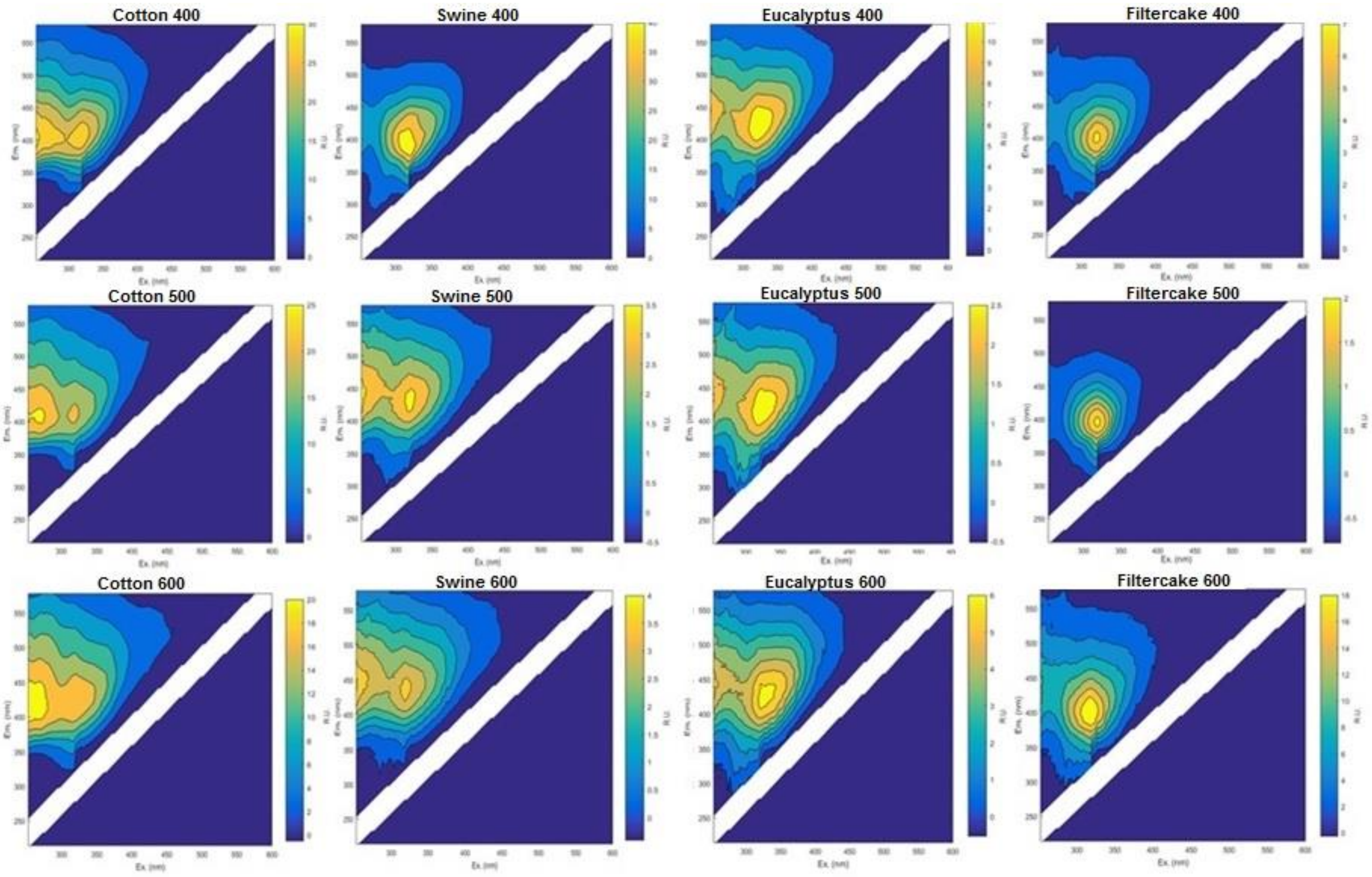
Appendix Figure H.1. Correlation between fluorescence index (FI), biological index (BIX), and humification index (HIX) with C/N ratio. Correlations were not significant, but display possible trends.

Appendix I Results of PARAFAC analysis

I.1 Examples of EEMs of DOC in leachate from different biochar feedstocks

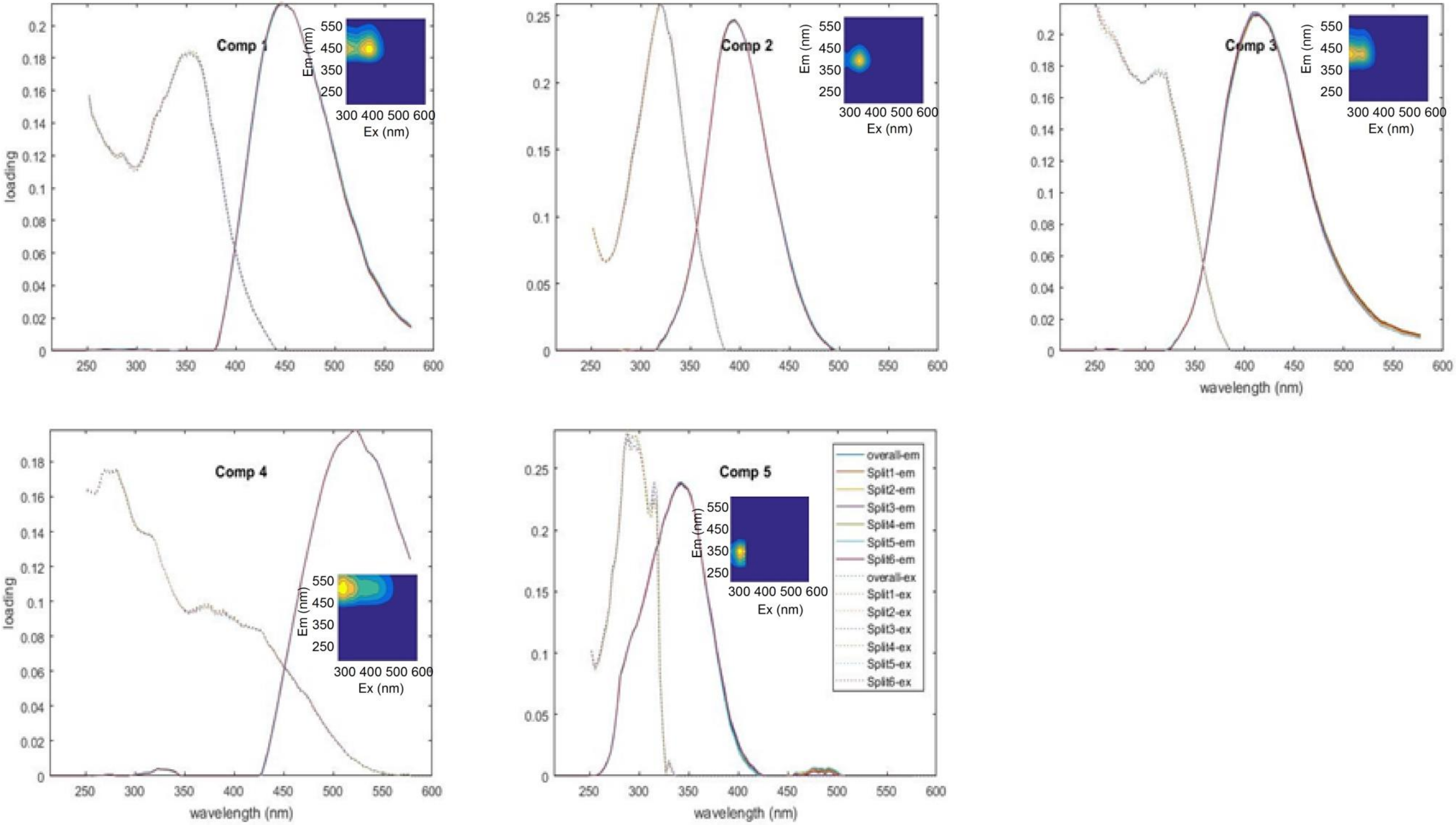


Appendix Figure I.1. Example of EEMs for control treatment (no biochar)



Appendix Figure I.2. Examples of EEMs for each of the biochar treatments

I.2 Overlaid spectra of a 5-component model



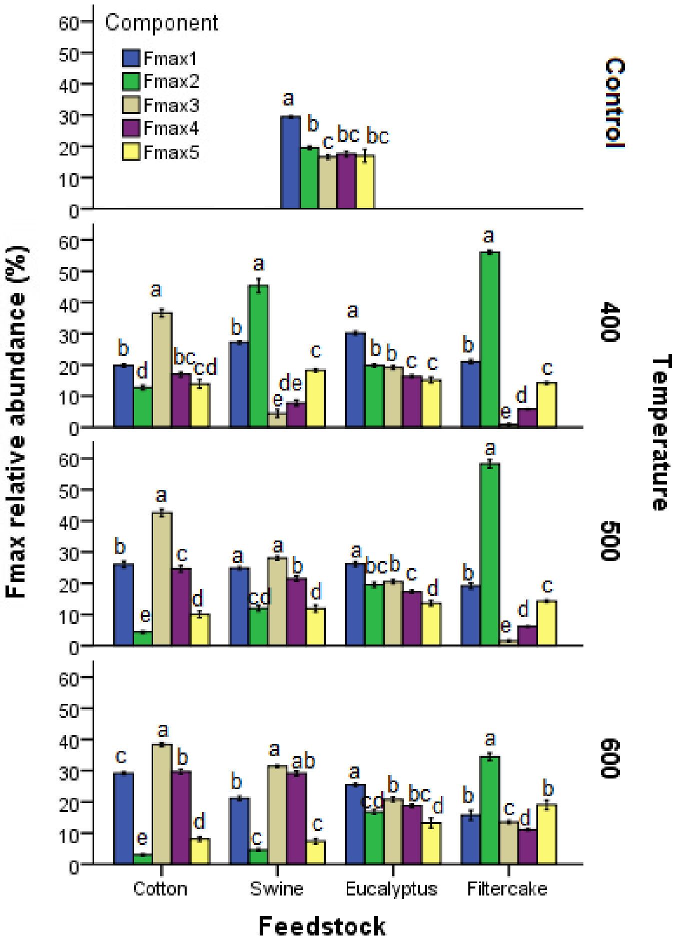
Appendix Figure I.3. Overlaid spectra of a PARAFAC 5-component model validated with 3 split comparisons, showing 6 unique splits vs overall model (*n* = 282).[a]

[a] In split-half validation, a number of models are made and compared after splitting the data in half in several ways, providing a powerful way for validating models (Murphy *et al.*, 2013). Core consistency can also be used to select the best model, but it may protect too much against over-fitting (Murphy *et al.*, 2013). Models for SOM with 2- to 4- components tend to have high core consistencies (closer to 100%) while models with 5- or more components have core consistencies closer to zero or even negative; yet the higher component models may still be showing actual chemical activity (Murphy *et al.*, 2013). In addition, different software can produce different core consistency values since different methods can be used; hence it is not recommended to use core consistency as the sole means of determining the number of components for interpreting EEMs (Uchimiya *et al.*, 2016). Therefore, although the core consistency for the 5-component model was low (7%) compared to that of the 2-component model (92%), based on other PARAFAC analysis of SOM (Erich *et al.*, 2012; Uchimiya *et al.*, 2013; Jamieson *et al.*, 2014), it was believed a split-half validated 5-component model, rather than a 2-component model, would be most appropriate for describing the chemical activity of DOC in this experiment.

Appendix Table I.1. Fmaxs for a PARAFAC 5-component model for biochar treatments and control (mean±1 SE).

	Fmax (Raman units)				
Biochar	1	2	3	4	5
Control	376.4±84.2	232.3±47.1	215.1±49.1	242.9±65.6	179.9±31.2
Cotton400	938.0±158.9	622.8±105.9	1557.0±238.4	805.9±128.2	697.0±174.6
Cotton500	1109.4±167.2	266.1±33.6	2219.2±294.1	1518.4±229.7	529.5±94.7
Cotton600	881.8±129.2	116.4±18.27	1537.2±211.4	1284.2±192.4	314.7±53.7
Swine400	3250.4±430.8	6094.0±813.2	488.9±120.6	1007.6±139.5	2415.1±329.3
Swine500	633.0±66.0	285.1±41.5	661.5±66.8	526.7±55.9	294.9±45.8

	Fmax (Raman units)				
Biochar	1	2	3	4	5
Swine600	863.2±109.9	135.8±21.1	993.1±121.4	960.6±119.2	218.7±33.4
Eucalyptus400	392.2±47.3	261.7±33.4	256.7±30.0	216.9±25.8	201.4±25.4
Eucalyptus500	379.4±58.7	239.9±37.0	276.1±42.2	230.8±35.4	166.1±22.8
Eucalyptus600	495.2±66.7	253.7±31.2	359.7±52.5	315.2±42.7	220.8±38.9
Filtercake400	2171.2±356.5	4894.6±749.6	20.1±38.5	526.0±82.7	1252.4±183.9
Filtercake500	1044.8±158.5	2817.4±355.4	81.6±21.1	323.7±49.1	731.3±99.2
Filtercake600	286.5±60.8	420.7±80.5	180.2±45.2	145.9±32.6	240.8±42.8



Appendix Figure I.4. Fmax relative abundance (%) of each component (Components 1-5) per treatment. Different letters indicate significant differences between components for each treatment (Games-Howell test; *P* <0.05).