CLOSING THE CARBON LOOP IN SUGARCANE BIOETHANOL: EFFECTS OF FILTERCAKE BIOCHAR AMENDMENT ON SOIL QUALITY, LEACHING AND CARBON UTILIZATION

by

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Abstract

Commodity prices and rural development in Brazil are driving the rapid conversion of the Cerrado biome, a highly diverse ecosystem with nutrient-poor soils. The expanding agricultural footprint includes sugarcane for bioethanol production, which boasts one of the highest net energy yields of commercial biofuels. However, energetic assessments fail to consider ecosystem costs, including soil degradation and impacts on water quality through the release of organic effluents. This thesis examined the use of charcoal or *biochar* made of 'waste' biomass (filtercake) as a soil amendment to reduce soil carbon (C) loss and water quality impairment. The effect of biochar on the leaching of a liquid waste with high eutrophication potential, vinasse, was also examined. Soil amendment with filtercake biochar improved soil pH, cation exchange capacity (CEC), nutrient availability (P, K, Mg, Ca, Mg, Fe, Mn, and Zn), and water retention. Amendment with filtercake biochar rather than raw filtercake also greatly decreased CO_2 loss to rapid mineralization. Furthermore, biochar amendment decreased the loss of dissolved organic carbon (DOC) from a cultivated Ferralsol, with or without co-application of vinasse, through preferential retention of larger and more complex, humified DOC species. In contrast, biochar did not attenuate nitrate (NO_3^-) leaching. Finally, $\delta^{13}C$ isotope analyses were used to examine the effect of raw *vs.* pyrolyzed residues on C turnover in an uncultivated soil, which suggested that whereas raw filtercake appeared to be mineralized preferentially over native soil organic carbon (SOC), biochar application appeared to provoke mineralization of native SOC. Overall, this project suggest that filtercake biochar may represent a valuable opportunity to better manage solid and liquid organic agricultural wastes in bioethanol production, with the potential to close nutrient loops and improve soil quality. However, further work is required to better understand the effect of filtercake biochar on soil C turnover and its long-term stability.

Preface

Chapters 1 and 5 are the sole work of the author. The data were collected from the literature and portions of these chapters will be combined to create a publishable paper regarding the potential for biochar use in the sugarcane bioethanol industry. For these and all other chapters, writing was the sole responsibility of the author.

Chapter 2 was a collaborative effort that has been published (Eykelbosh, A.J., Johnson, M.S., Santos de Queiroz, E., Dalmagro, H.J., Guimarães Couto, E., 2014. Biochar from sugarcane filtercake reduces soil CO_2 emissions relative to raw residue and improves water retention and nutrient availability in a highly weathered tropical soil. PLoS One, 9(6), e98523). Briefly, the author carried out the laboratory experiments at the Universidade Federal de Mato Grosso (UFMT, Cuiabá, Brazil), with the assistance of Edmar Santos de Queiroz and Higo Jose Dalmagro. Drs. Mark Johnson and Dr. Eduardo Couto contributed to data analysis and critical revision of the manuscript. A number of the analyses described in the paper were performed outside of the laboratory. We thank Dr. F. Lobo for providing laboratory space at UFMT, Dr. L. Lavkulich for providing Raman spectrographs, Dr. F. Unda for performing the carbohydrate analysis, and Dr. H. McLaughlin for advice on benchtop biochar production. We would also like to thank the faculty and staff of the Programa de Pós-Graduação em Física Ambiental at UFMT for assistance in sourcing materials and equipment loans and the Laboratório de Caracterização em Novos Materiais (LACANM) for SEM analysis.

Chapter 3 has been developed as a manuscript and accepted for publication by the *Journal of Environmental Management*. This work was carried out at UFMT, in Cuiabá, Brazil. The author was responsible for experimental design and carrying out the analyses, with the assistance of Edmar Santos de Queiroz and João Angelo Sguarezi de Figueiredo. Dr. Mark Johnson and Dr. Eduardo Couto contributed to the data analysis and critical revision of the manuscript. Special thanks are owed to Ashlee Jollymore for assistance with fluorescence and absorbance spectrometry, and to Dr. F. Lobo for providing laboratory space and equipment.

Chapter 4 was designed by the author and carried out with the assistance of Edmar Santos de Queiroz and Heiriane Martins and data analysis was carried out by the author with the contribution of Drs. Mark Johnson and Eduardo Couto. Samples subjected to isotopic analysis were processed at Centro de Energia Nuclear na Agricultura (CENA) at the University of São Paulo. Work space and equipment was provided by Dr. Daniela Campos (UFMT).

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CEC – cation exchange capacity

CFE – chloroform fumigation–extraction

CFI – chloroform fumigation–incubation

DOC – dissolved organic carbon

EEMs – excitation-emission matrices

FI – fluorescence index

FrI – freshness index

GHG – greenhouse gas

HI – humification index

HTT – highest heating temperature

MRT – mean residence time

NEROI – net energy return on investment

NO_3^- – nitrate

NPOC – non-purgeable organic carbon

p-*NP* – *para*-nitrophenol

PPO – polyphenol oxidase

RU – Raman Units

SOC – soil organic carbon

SOM – soil organic matter (~58% carbon)

$SUVA_{254}$ – specific UV absorbance at 254 nm

TRFLP– terminal restriction fragment length polymorphism

Glossary

Acetate – An anion with the chemical formula $C_2H_3O_2^-$ that is important in various biochemical reactions.

Aconitate – An anion with the chemical formula $C_6H_3O_6^{3-}$ that is important in various biochemical reactions.

Acrisol – Soil group characterized by a clayey subsoil with low cation exchange capacity in the World Reference Base for Soil Resources.

Arenosol – Soil group characterized by extremely sandy texture within the World Reference Base for Soil Resources.

Bagasse – the fibrous sugarcane residue remaining after milling, during which the sugarcane is crushed and the juice collected.

Chemoheterotrophic – referring to organisms that use organic compounds for both energy and for the synthesis of other organic molecules.

Dystrophic – Referring to soils with low nutrient content.

Ferralsol – Soil group characterized by the presence of kaolinite and sesquioxides.

Fertigation – fertilization combined with irrigation.

Filtercake – the sludge remaining after the sugarcane juice is clarified through the addition of $CaOH_2$; the precipitate (sludge or filter mud) is removed through filtration.

Humification – the transformation of organic substances from identifiable plant-derived components to amorphous organic material

Mineralization – the biotic or abiotic transformation of elements bound as organic molecules into their inorganic constituents.

Oxalate – A dianion with the chemical formula $C_2O_4^{2-}$ that is important in various biochemical reactions.

Photoautotrophic – referring to organisms that use light as their source of energy and fix carbon for the synthesis of organic molecules.

Stabilization – the physical and chemical processes that protect organic materials from decomposition.

Tyrosine – A polar amino acid with the chemical formula $C_9H_{11}NO_3$ that is important to a variety of biosynthetic pathways.

Vinasse – the liquid effluent remaining after ethanol has been recovered through distillation of the fermented sugarcane wine.

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Finally, I would like to thank my husband Leo, who knows what he did.

Chapter 1: Introduction

1.1 Biofuels, land conversion and the creation of 'carbon debts'

Biofuels are expected to fulfill dual roles in both energy security and climate mitigation. In Brazil, long-term investment in the sugarcane cultivation and bioethanol production, alongside rising oil prices, have combined to create a thriving bioethanol industry that accounts for 27% of current global production (Renewable Fuels Association, 2013). Previous studies have demonstrated that the net energy return on investment (NEROI) for Brazilian bioethanol is excellent, producing roughly 9–10 units of energy for every unit of fossil fuel energy invested; this is in contrast to values from 0.8–1.6 for corn ethanol (Farrell et al., 2006). With further technological advances, this value is expected to increase to 11.2, driven by further reductions in fossil fuel inputs (esp. fertilizer usage) and increased bioelectricity production to displace fossil power (Macedo et al., 2008). At present, biomass electricity, predominantly derived from the combustion of sugarcane residue (bagasse), makes up 6.8% of Brazil's domestic electricity supply (EPE, 2013).

In contrast to the demonstrated energetic benefits of Brazilian bioethanol, the overall environmental impact of this product, in terms of mitigating climate change through reduced carbon (C) emissions and other impacts, remains unclear. Current life cycle analyses show that sugarcane bioethanol use in Brazil is associated with large avoided CO_2 emissions (Galdos et al., 2010; Lisboa et al., 2011; Macedo et al., 2008; Pacca and Moreira, 2009). In one estimate, bioethanol and bioelectricity from sugarcane avoids the emission of 25.8 million tonnes of CO_2 equivalents per year in Brazil (Macedo et al., 2004). These studies account for most fossil fuel

inputs and greenhouse gas (GHG) emissions directly associated with sugarcane cultivation, processing, transport, and end use. However, the acknowledged limitation of these studies is the exclusion of C and GHG fluxes that are one or two steps removed from the agro-industrial process, namely the effects of direct and indirect land use change and management effects after conversion to agriculture. Exclusion of these factors may lead to overestimation of the climate-mitigating potential of bioethanol, and mask other evidence of poor environmental performance, such as soil degradation and subsequent threats to soil ecosystem services and productivity.

In Brazil, for example, most future sugarcane expansion will occur in the savannah or *Cerrado* biome of the center-western region (Martinelli and Filoso, 2008). Since 2008, this region has seen a 107% increase in land used for sugarcane cultivation, rising from 873,274 to 1,810,830 ha of sugarcane harvested (SIDRA, 2014). Although in some respects ideal for large-scale mechanized agriculture (Goedert, 1983), this region is also rich in biodiversity and has significant above- and below-ground C storage as biomass and soil organic carbon (SOC) (Jimenez and Lal, 2006). When Cerrado is converted to sugarcane cultivation, above-ground biomass is burned or used in forest products; below-ground C is also affected by changes in plant C inputs and increased soil respiration due to cultivation. For Cerrado soil, conversion to sugarcane is associated with a "debt" of lost C that required at least 17 years of subsequently avoided CO_2 emissions (through bioethanol use) to "repay"; this repayment time increases to 319 years if tropical forest is removed (Fargione et al., 2008). Post-conversion, SOC cycling and related GHG production (CO_2, CH_4, and N_2O) can be positively or negatively affected by management practices, including fertilization, liming, tillage, and pre-harvest burning (Carmo et al., 2013; Cerri et al., 2011; La Scala et al., 2006; Lisboa et al., 2011; Oliveira et al., 2013).

However, overall, converting native ecosystems to sugarcane cultivation without any additional management results in drastic and, for the most part, persistent decreases in SOC to 1 m[1] (Oliveira et al., 2010; Resende et al., 2006; Vasconcelos et al., 2010).

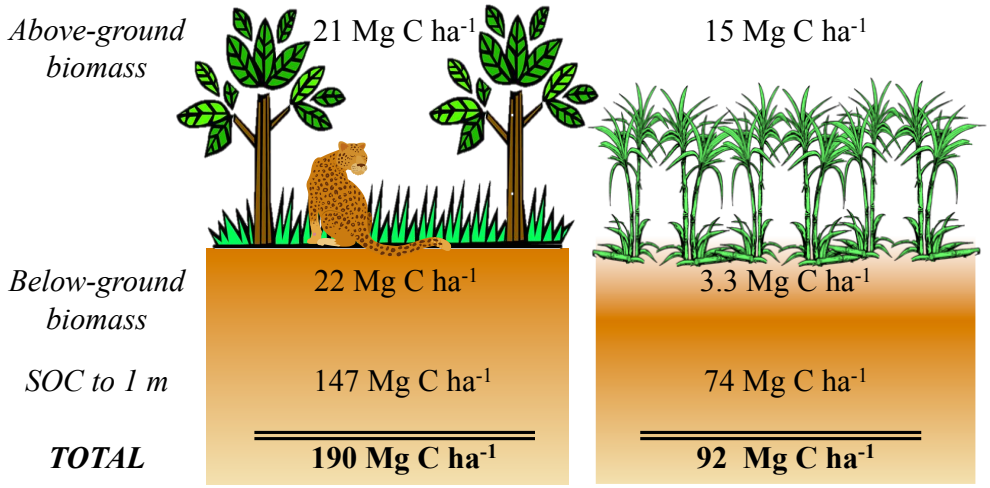

Figure 1.1. Carbon debt incurred when Cerrado *sensu stricto* is converted to sugarcane. Values represent means taken directly from the literature for above-ground biomass C (Abdala et al., 1998; Barbosa and Fearnside, 2005; Castro and Kauffman, 1998; Lilienfein et al., 2001; Ottmar et al., 2001), below-ground biomass C (Abdala et al., 1998; Castro and Kauffman, 1998; Lilienfein et al., 2001), and soil organic C (to 1 m) (Abdala et al., 1998; Brossard et al., 1997; Chapuis Lardy et al., 2002). For sugarcane C stocks, above- and below-ground stocks were derived based on published estimates for the allocation of photosynthetically fixed carbon to above *vs.* below-ground plant parts (Resende et al., 2001), as calculated in Appendix A. SOC to 1 m for sugarcane was calculated as a 50% decrease of the native pool, based on literature showing SOC decreases of 40–58% when native forest or Cerrado is converted sugarcane (Galdos et al., 2009; Oliveira et al., 2010; Vasconcelos et al., 2010; Vitorello et al., 1989).

[1] To minimize costs and maximize coverage, soil surveys typically limit soil sampling to the most superficial levels of the soil (0 to 100 cm). Ideally, however, SOC would be analyzed to the regolith, the unconsolidated rock and debris that serve as parent material for soil formation. This is because, in tropical regions with very deep soil profiles (up to 20 m in some regions), examining SOC in only superficial layers may greatly underestimate total carbon storage (Jimenez and Lal, 2006). This may be particularly important in deep Cerrado soils, where SOC to 1 m was found to represent only 32% of total SOC to 6.2 m (Abdala et al., 1998). Nevertheless, SOC to 1 m remains a relevant indicator of soil quality change because it is these most superficial layers that are most strongly and immediately affected by changes in management.

From the literature on Cerrado ecosystem C, we can see that conversion to sugarcane results in a C debt of roughly 92 Mg C ha^{-1} (**Fig. 1.1**). Because organic matter is critical to nutrient cycling and sustained fertility in highly weathered, rapidly draining tropical soils (Tiessen et al., 1994), the effects of post-conversion agricultural practices on soil C and soil quality should be weighed heavily when assessing the overall ecological impact of sugarcane bioethanol.

1.1.1 Carbon cycling and crop residues in sugarcane cultivation

The first step toward improving C management is to understand C flows in sugarcane fields. At present, there is no widely accepted C budget for Brazilian sugarcane, and very little previous work on ecosystem C exchange. For modeling purposes, however, a set of assumptions have been used in which net annual C fixed via photosynthesis is allocated or partitioned among the leaves and flags (30%), stalks (47%), and roots (22%) of the plant (Resende et al., 2001). Thus, starting from dry harvested biomass (stalks), it is possible to estimate the mass of C that is fixed on site and then either exported, burned, or allowed to decompose on the field (**Fig. 1.2**). In addition to C input by leaf litter, roots, and root exudates, a portion of the harvested biomass is returned to the field after processing, as a means of soil amendment and waste management. These organic residues are *vinasse*, a liquid waste rich in dissolved organic carbon (DOC) that is produced during distillation, and *filtercake*, a solid waste produced during sedimentation. These two residues, when added back to the same field from which they were produced, make up 15%

of soil C input on a per hectare basis, or 30% of C input where pre-harvest burning is used.[2] Thus, the total annual C input to soil for a sugarcane field is roughly 5–10 Mg C ha^{-1} y^{-1}.

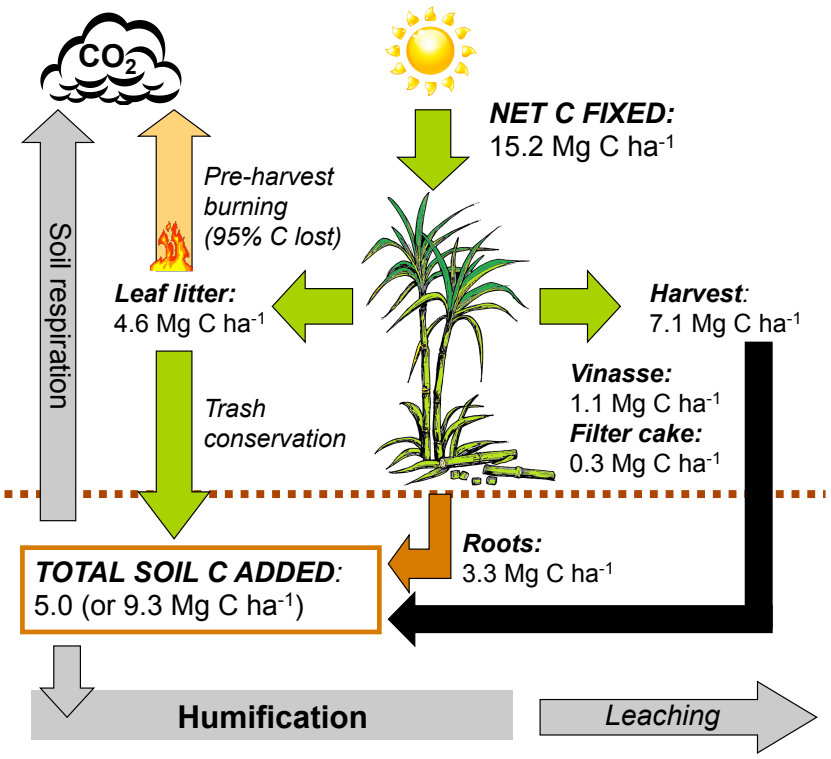

Figure 1.2. Estimated annual carbon flows in a hectare of sugarcane.

Carbon inputs for each element were calculated as described in Appendix A. Please note that bagasse is not included in this budget because it is not returned to the field, but is rather burned for cogeneration.

This estimated C budget gives an idea of the relative importance of organic residues in C cycling in sugarcane fields. Indeed, these combined carbon flows are much higher than those estimated for native Cerrado, where litter fall is much lower (~0.2–2 Mg ha^{-1} y^{-1}) and litter and root turnover is on the scale of years (1.1–9.9 and 9–12 years, respectively) (Bustamante et al., 2012;

[2] It should be noted however, that residues are not distributed equally among cane fields; fields close to the distillery typically receive large inputs (up to 4.7 Mg vinasse C ha^{-1} and 2.3 Mg filtercake C ha^{-1}), whereas distant fields may receive nothing.

Bustamante and Ferreira, 2010) rather than months. Despite this relatively large input, vinasse and/or filtercake application remains insufficient to restore SOC to native ecosystem levels after land conversion (Oliveira et al., 2010; Resende et al., 2006; Vasconcelos et al., 2010). Only one study has shown recovery of C stocks to near pre-disturbance levels after 18 years of cultivation, but this occurred under vinasse and filtercake application rates well above the legally mandated limit for protecting nearby water sources (Silva et al., 2007). The inability to retain soil C derived from inputs has been attributed to the practice of deep soil tillage or sub-soiling (tillage 50–75 cm deep) that occurs before re-planting at the end of a six-year growth cycle (Resende et al., 2006). This decrease in soil C storage under sugarcane cultivation, even with ample organic amendment, represents a problem for both biofuel sustainability and soil health.

1.2 Fate of vinasse- and filtercake-derived carbon

There are several possible fates for the residue-derived C as it decomposes in cane fields. Broadly, these are stabilization, mineralization, or transport **(Fig. 1.3)**. *Stabilization,* which refers to the physical and chemical processes that protect organic materials from decomposition, is primarily achieved through adsorption to clay minerals (chemical stabilization), incorporation into soil aggregates (physical stabilization), and biochemical protection due to the material's original chemical recalcitrance or that which is acquired through humification[3] (Six and Conant, 2002). *Mineralization* refers to the biotic or abiotic transformation of elements bound as organic molecules into their inorganic constituents, which may be either taken up by plants (NO_3^-) or lost to the environment (CO_2) (Guggenberger, 2005). When fresh, unprotected organic matter is

[3] *Humification* refers to the transformation of organic substances from identifiable plant-derived components to amorphous organic material (Guggenberger, 2005), and is most strongly influenced by the physical and chemical environment, the chemical composition of the starting material, and the microbial community.

added to the soil, the degree to which this material may be mineralized or stabilized depends on both the receiving environment (*e.g.*, presence of clay minerals, the microbial community present) as well as the material's chemical nature and subsequent bioavailability.

Vinasse and filtercake differ markedly in this respect. Sugarcane vinasse is composed of roughly 80–90% dissolved organic carbon (DOC) as organic acids (*e.g.,* aconitate, acetate, and oxalate) and simple sugars (sucrose and glucose); the remaining 10–20% is made up of particulate organic matter, being mostly cellulose and hemicellulose (Benke et al., 1998; Doelsch et al., 2009). Accordingly, vinasse C is highly bioavailable and likely to be mineralized rapidly in the soil, which is supported by previous studies showing large but transient soil CO_2 effluxes (Santos et al., 2009a) and increases in microbial biomass (Santos et al., 2009b) in response to vinasse application.[4] Although there is evidence to suggest that long-term (35 years) vinasse application increases humic substances in the soil, including the fulvic acid, humic acid, and humin fractions, these changes did not affect the overall degree of humification (Canellas et al., 2003). Filtercake, like vinasse, contains a large amount of bioavailable C in the form of cellulose and hemicellulose, but contains a much larger proportion of recalcitrant C in the form of lignin (George et al., 2010) and humin (Busato and Zandonadi, 2010). Therefore, although filtercake application to soil increases CO_2 efflux (Carmo et al., 2013; Rasul and Khan, 2008), it may contribute to the biochemically protected, humified fraction to a greater degree than vinasse.

[4] In addition to CO_2 efflux, high-volume vinasse C applications that create saturated, anaerobic soil conditions with low redox potential may also promote the production of other greenhouse gases (GHGs) (Masscheleyn et al., 1993). Vinasse application may further promote methanogenesis due its enrichment in acetate, a preferred substrate for methanogens (Sugimoto and Wada, 1993). Significant N_2O fluxes have also been observed in response to vinasse field applications, although CH_4 emissions were low (Carmo et al., 2013). Nevertheless, these potential fluxes remain important due to the high global warming potential (GWP) of CH_4 and N_2O, which are 25 and 298 times that of the same amount of CO_2 over 100 years (Forster et al., 2007).

These differences in the bioavailability to microorganisms may have effects on soil C cycling when filtercake is applied alone or in combination with vinasse. Previous work has shown that the addition of fresh labile C, rather than "topping up" stored C, may provoke or prime the turnover of more stable C pools, which may ultimately limit the growth of soil C stocks or lead to C depletion (Fontaine et al., 2007). These *priming effects* are thought to be mediated by a succession of microbial communities that potentiates the decomposition recalcitrant C. In general, it is assumed that fresh C additions stimulate the activation and turnover of dormant bacteria, which quickly deplete the easily available C by absorbing simple compounds and metabolizing them internally, expanding their own biomass. After a slight lag, these rapid responders are followed by bacteria and fungi that preferentially utilize more recalcitrant C (Kuzyakov, 2010). Fungi, in particular, are thought to feed off the bacterial necromass and more stable soil C through the production of extracellular enzymes that remain active independently in the soil for some time. Fungi also show extensive hyphal growth throughout the soil environment, giving them much greater spatial influence than bacteria (Blagodatskaya and Kuzyakov, 2008). Thus, the dynamics of microbial communities are a key driver in regulating soil C turnover (Gleixner, 2013).

Given this dynamic, it is possible that C cycling in sugarcane fields is affected by the "how" of organic residue application. Vinasse is typically applied as it is produced to recently harvested fields, and often in excess (Martinelli and Filoso, 2008; Prado et al., 2013). If vinasse is applied to bare soil at the beginning of the harvest sequence, it represents a sudden input to soils that have been largely depleted after a period of exposure with no plant input. Under such conditions,

vinasse application may cause the type of booming microbial succession described above, which may result in the mobilization of stabilized soil C, either at the surface or deeper in the soil profile. Filtercake is also turned or harrowed into the soil at the beginning of the growth cycle, and although filtercake also stimulates soil microbial activity and soil CO_2 efflux (Rasul and Khan, 2008), the large fraction of much more resistant C may help to modulate the soil microbial community over the season. If so, coordinating or combining vinasse and/filtercake application may be a means to manipulate the ratio of labile to recalcitrant C and C utilization in the field over time. More specifically, it is important to understand how vinasse and filtercake additions, alone or in combination, affect C storage within this agroecosystem.

Finally, some soils may also promote the mobility of C and other dissolved species present in vinasse, representing both a loss in terms of soil nutrients as a well as a potential threat to nearby aquatic ecosystems. In the Brazilian Cerrado, electrostatic interaction among iron and aluminum oxides and kaolinite, and the activity of soil fauna, contribute to a very strong microaggregate structure in the predominant Ferrasol soils (Balbino et al., 2002; Neufeldt, 2002). This characteristic favours mechanized agriculture because these microaggregates are highly resistant to compaction, and they also promote rapid drainage (Goedert, 1983). In addition, the predominant clay minerals in Ferralsols are kaolinite, gibbsite ($AlOH_3$), and goethite (FeOOH) (Van Wambeke, 1974), low-activity clays that are consequently less effective in retaining the high concentrations of cations found in vinasse (Doelsch et al., 2009). Excessive application, therefore, may allow vinasse constituents to move rapidly through the soil profile and perhaps leach to groundwater or move laterally to nearby streams. Although terrestrial C and nutrient inputs are vital to aquatic ecosystems, the large amounts of sugarcane residues produced and

applied have previously and continue to cause problems with eutrophication (Martinelli and Filoso, 2008), with effects on water chemistry and aquatic organisms at the catchment level (Gunkel et al., 2006; Ometo et al., 2000). Leaching of vinasse and the contamination of ground and surface water are also a human health risk, due to the potential for the production of DOC-derived carcinogenic compounds when water is chlorinated for human consumption (Volk et al., 2005).

1.3 Comparing current practice with a low-tech alternative: filtercake biochar

Given that maintaining SOC levels is necessary for soil fertility, several techniques have been widely researched to achieve this. These include organic waste application, as in sugarcane cultivation, as well as no-tillage, residue retention, and the use of biochar. *Biochar* is produced when biomass is heated above 500°C in the near absence of oxygen, allowing thermochemical decomposition with limited CO_2 emission. Under optimal conditions, the resulting porous, friable charcoal retains most of its C as aromatic C and is consequently much more resistant to microbial decay (Krull et al., 2009). In native Cerrado, a fire-adapted ecosystem, charcoal or black C is a critical stable component of total soil C and has been identified as a resilient soil C fraction during land use change (Abdala et al., 1998; Roscoe et al., 2001). Thus, incorporating charred biomass into soils as biochar has been proposed as a means by which biofuel production might cover C deficits generated during land conversion and become truly C negative (Laird, 2008). In Japan, a lifecycle assessment accounting for CO_2 emissions from the production, transport, and application of bagasse biochar (*vs.* returning raw bagasse to the field) found that pyrolyzing cane residues before incorporation would allow the sequestration of an additional 60–90 Mg CO_2 ha^{-1} at an application rate of 3% d.w. biochar (Kameyama et al., 2010).

Beyond C sequestration, biochar may also have important soil quality and crop productivity benefits, particularly for rapidly draining, nutrient-poor Cerrado soils. A previous meta-analysis showed that biochar improved soil water retention, nutrient retention, and plant productivity, particularly in coarse, acidic soils (Jeffery et al., 2011). Biochar may also have potential benefits in terms of air and water quality, as it has been shown to reduce GHG emissions from the soil surface and prevent pesticide and DOC leaching through the subsoil (Novak et al., 2010; Spokas and Reicosky, 2009; Spokas et al., 2009). Biochar may also help to counter anticipated water deficits as the Cerrado's climate becomes markedly warmer and drier (Hoffmann and Jackson, 2000). At the microbiological level, it is thought that biochar increases the available range of microhabitats while also stabilizing organic substrates through adsorption; this is postulated to promote microbiological diversity while modulating or restricting activity (Thies and Rillig, 2009). Thus, increasing the fraction of black C in soil may have numerous benefits for sugarcane cultivation, while also addressing the key issue of C loss due to potential priming effects and leaching.

Previous studies on sugarcane biochar have used *bagasse*, the fibrous by-product left over after crushing and pressing the cane, as feedstock. Feedstocks high in lignin, like bagasse, tend to yield larger amounts of biochar with higher C sequestration potential (Demirbas, 2004; Lee et al., 2013). Bagasse biochar has also been shown to increase soil moisture, sugarcane yields, and sugar content, while decreasing soil dry density and percolating/leachable nitrate (Chen et al., 2010). However, the fundamental problem with using bagasse for biochar is that it is a valued by-product. At Brazilian bioethanol plants, bagasse produced locally is burned to generate

bioelectricity that is used to operate the distillery. Surplus energy is sold back into the grid as an extra source of revenue and represents a large proportion of biomass energy used in Brazilian (EPE, 2013). The demand for bagasse may further increase as cellulosic biofuel technology develops (Cardona et al., 2010). Thus, deviating bagasse to produce biochar may be neither practical nor desirable. In contrast, filtercake is a nuisance by-product for distilleries. Not only does it have a very unpleasant odour, but its high water content (70%) increases transportation costs for disposal as a soil amendment, and when left to decompose in heaps it represents a significant eutrophication risk to nearby streams (George et al., 2010).

Although filtercake is primarily viewed as a waste disposal problem, its high cellulose (42%) and lignin (15%) contents (Benke, 1998) recommend it as another potential biochar feedstock. However, no information is currently available regarding the effects of filtercake biochar on soil quality. More specifically, it is unknown how raw *vs.* pyrolyzed filtercake may affect water, nutrient, and C retention in sugarcane fields, with or without co-application with the much more mobile vinasse.

Furthermore, it is unclear whether filtercake biochar would amplify rather than ameliorate soil C priming and storage relative to the application of raw residues, as currently practiced. As mentioned, it is possible that the application of vinasse in particular may prime more stable C for decomposition; however, previous studies have shown that freshly added labile C and biochar C may also interactively prime each other, leading to increased mineralization of both C sources than is observed when either is added alone (Hamer, 2004; Keith et al., 2011; Zimmerman et al., 2011). The magnitude of this priming potential varies greatly with the type of feedstock used, the

method of production, and the organic matter status and pH of the soil examined (Cross and Sohi, 2011; Luo et al., 2011; Zimmerman et al., 2011). Thus, investigation specific to typical Cerrado soils and the specific feedstock (filtercake) are required to examine the effects of biochar additions and compare them to current practices.

1.4 Research questions

To better understand the contribution and fate of raw sugarcane residue to C cycling in sugarcane cultivation, and how those inputs could be better managed to increase C storage and soil quality under sugarcane cultivation (*i.e.*, co-application or pyrolysis of residues), this thesis used laboratory studies to examine the following four questions:

1. What are the effects of pyrolyzed filtercake residue on soil quality in terms of commonly assessed agronomic characteristics such as pH, cation exchange capacity, water retention, macro- and micronutrient availability, CO_2 efflux, and the bioavailability of added C (**Chapter 2**)?
2. How does the presence of biochar affect the mobility of vinasse constituents and the stabilization of DOC in a cultivated soil (**Chapter 3**)?
3. How does the application of raw filtercake or filtercake biochar differentially affect soil C utilization and CO_2 effluxes when applied alone or in combination with vinasse (**Chapter 4**)?
4. How does the application of raw filtercake or filtercake biochar affect the abundance and activity of soil microbial communities when applied alone or in combination with vinasse (**Chapter 4**)?

1.5 Significance

In the Brazilian Cerrado, which is anticipated to be the site of most future sugarcane expansion (Cerqueira Leite et al., 2009), a large portion of ecosystem C is stored below ground (Batlle-Bayer et al., 2010). However, if sugarcane expansion for bioethanol development continues at its current pace in center-western Brazil, land conversion could provoke a substantial C release from the Cerrado biome. Furthermore, given predictions for a warmer, drier future climate on the Cerrado (Hoffmann and Jackson, 2000), this loss of soil C may equate with a decrease in available soil water that threatens productivity. For these reasons, it is critically necessary to evaluate new strategies for C management in sugarcane cultivation.

This proposal outlines a series of research questions aimed at characterizing a new potential tool for C management in sugarcane bioethanol (filtercake biochar) and better understanding the fate and effects of this and other carbonaceous residues applied to sugarcane fields. Furthermore, this thesis explored the potential use of biochar as a means of improving C sequestration and soil quality without disrupting waste management activities or deviating valuable feedstocks. The unique contributions from the proposed research include the very first attempt to produce and characterize biochar from filtercake, a nuisance by-product, and to evaluate the effect of this biochar on soil C cycling in comparison to disposal as raw organic waste, the current industry standard. This thesis also incorporates a leaching study with a isotopic C utilization study, providing a more integrated understanding of C flux between biotic and abiotic components of the soil.

Chapter 2: Biochar from sugarcane filtercake reduces soil CO_2 emissions relative to raw residue and improves water retention and nutrient availability in a highly weathered tropical soil

2.1 Introduction

The principal challenge for agricultural sustainability and feeding an ever-growing population is avoiding the degradation of soil, a vital but slowly renewable resource. In Brazil, economic and social drivers have come together to drive agricultural expansion into pristine regions (Martinelli et al., 2010). In addition to land use change in the Amazon and Atlantic forests, the savannah or Cerrado of Brazil's center-west has come under intense conversion, with an estimated 50% of this highly biodiverse region already converted to cropping and pasture, primarily within the last 40 years (Klink and Machado, 2005). Conversion has increased the risk of soil degradation through soil organic carbon (SOC) loss, deteriorating soil structure, and increased risk of erosion (Sparovek et al., 2007), with knock-on effects for other ecosystem services.

Given that agriculture is central to Brazil's economic development, and that agricultural products from this region will help to feed and fuel the world, innovative agricultural technologies and practices are critically required to prevent soil degradation. Sustainable practices, such as conservation agriculture, have and will continue to play an important role (Machado and Silva, 2001), but supplementary strategies have also been proposed. One of these is to enhance soil storage of black carbon (C), which plays an important role as a geosorbent and microhabitat (Cornelissen et al., 2005), a modifier of soil chemistry and C cycling (Liang et al., 2010, 2006),

and as a slowly available C source for microbial activity (Czimczik and Masiello, 2007). In natural soils, elevated black C has been associated with increased fertility, water retention, and more rapid incorporation or stabilization of new C inputs (Glaser et al., 2001; Liang et al., 2010, 2006). In undisturbed Cerrado ecosystems, black C produced through natural fires makes up a small but stable pool within the total soil C stock; however, this stock is depleted after disturbance (Roscoe et al., 2001).

Black C in soil can be enhanced through amendment with man-made charcoal or *biochar* derived from the pyrolysis of waste biomass, through which relatively labile plant-fixed C is transformed into a highly recalcitrant char. Although most work to date has focused on temperate soils, studies incorporating biochar into tropical soils have shown evidence of enhanced soil quality, nutrient availability, and productivity, and decreased leaching losses and acidification (Chen et al., 2010; Kameyama et al., 2012; Major et al., 2012; Steiner et al., 2007).

Sugarcane cultivation in particular may benefit from this approach because the industry produces several pyrolyzable residues. These include bagasse (crushed cane stalks), cane trash (leaves and stalk tips removed during harvest), and filtercake, a nutrient-dense sludge that is removed via filtration after the juice clarification step. Previous biochar studies on sugarcane residues have focused exclusively on bagasse, with positive results in terms of soil quality improvements and productivity (Chen et al., 2010; Kameyama et al., 2012). However, bagasse is a valuable by-product, both under current operations (*e.g.*, for cogeneration of heat and electricity in distilleries) and as a future lignocellulosic feedstock for second-generation biofuels (George et al., 2010). Thus, diverting bagasse for biochar production and soil amendment would come at the

cost of other gains, and as such bagasse biochar may be less likely to be implemented as a strategy for soil and C management.

Filtercake, in contrast, is a heavy, nutrient-dense residue that is sometimes spread on fields as a fertilizer (Prado et al., 2013), although the high biological availability of its components likely leads to the rapid mineralization and leaching losses common in tropical soils (Khalil et al., 2005). Filtercake management is hampered by its high water content, which makes it costly to transport and difficult to apply, as well as its potential contribution to nutrient runoff and eutrophication when over-applied (George et al., 2010). Thus, conversion of this highly labile residue into biochar may be an opportunity to turn a nuisance waste into a valuable soil amendment.

The objectives of this study were to first develop a benchtop reactor to produce biochar from sugarcane filtercake for research purposes and to examine its effects on soil quality. Filtercake biochar was subjected to physicochemical characterization and the effects of this product on soil pH, CEC, nutrient availability and water retention were compared to unamended soil. We also examined the effects of raw *vs.* pyrolyzed filtercake amendment on CO_2 efflux in combination with vinasse, a C-rich effluent resulting from distillation that is similarly disposed of via soil application (Prado et al., 2013) and may itself affect C cycling through priming of native soil C (Kuzyakov, 2010). These soil quality assays indicated that filtercake biochar may be useful as a soil C management tool with agronomic benefits for nutrient and water availability.

2.2 Materials and methods

2.2.1 Material collection and biochar production.

Permission to collect soil samples and organic residues (filtercake and vinasse) from private property was granted by the management of Usina Pantanal de Açúcar e Álcool (Ltda.) located in Jaciara, Mato Grosso, Brazil (15°55'28.11"S, 55°13'38.94"W, elevation 690 m). This region had a mean annual temperature of 25.5°C and mean annual precipitation of 1495 mm from 2000–2013 (BDMEP, 2013a). Soil was collected at the end of the dry season from a single soil pit in a sugarcane field that had been harvested in the previous month (no active plant growth). The mineral soil of the top 0–10 cm of the rhizosphere was collected and sieved to 2 mm; roots were removed by hand. The soil was red-yellow Ferralsol (FAO taxonomy) with a sandy clay loam texture (64% sand, 9% silt, 27% clay) and total carbon (C) and nitrogen (N) contents of 1.5% and 0.07% dry weight (d.w.), respectively. Soil pH (6.13 ± 0.03) was determined in the laboratory by mixing 3 g of soil in 27 g of water, followed by 30 min of intermittent shaking, 30 min of settling, and measurement with a handheld meter (HI 98121, Hanna Instruments, USA).

Vinasse was collected from canals close to the point of application and aliquots were frozen at –20°C until use (photo included in **Appendix B**). Vinasse total C concentration (1.29 g C L^{-1}) was determined via combustion of the lyophilized solid residue to obtain % C (CHN-1110 elemental analyzer, Carlo Erba Instruments, Italy) and then multiplied by total solids. Filtercake also was collected fresh from Usina Pantanal and dried at 45°C for four days in a forced-air oven. Because particle size affects final biochar characteristics (Demirbas, 2004), the size classes of dried filtercake particles subjected to pyrolysis were maintained constant across biochar batches. Briefly, dried material was gently crushed and sieved to collect size fractions of 2–4 and 4–10

mm. To promote clearance of gases from within the reactor, particles < 2 mm were not used. Approximately 850 g of this material (50% from each size class) was pyrolyzed in a custom-made benchtop biochar reactor. The reactor consisted of a 10- × 50-cm steel cylinder (diameter × length) closed on one end with a circular steel plate. The plate was perforated with a 1/4″-brass male compression fitting to serve as an inlet for N_2, which was used to purge the reactor of oxygen. The flanged open end was closed using a gasket made of high-temperature fibreglass cord and a steel plate held in place with a grooved circular clamp. The removable steel plate was perforated with two additional brass fittings, one for exhaust and a second to allow placement of a type-K thermocouple (Omega Engineering Inc., Stamford, CT) for monitoring internal temperature. These apertures were protected by a 1-mm stainless steel mesh at either end of the cylinder. Images of the reactor are presented in **Appendix B.**

This assembly was mounted inside a large Linn Elektric muffle furnace with a programmable controller (KK 260 SO 1060; Linn High Therm GmbH, Germany). The slow pyrolysis program was as follows: slow heating to 575°C at a rate of 5–6°C min^{-1}, holding at 575°C for 3 h, followed by slow cooling overnight to room temperature. The entire program was carried out under oxygen-limited conditions (N_2 purge, 0.5–1 L min^{-1}). The program was selected based on previous studies showing that this heating rate, pyrolysis temperature, and time were conducive to creating a biochar (from bagasse) retaining > 500 mg C g^{-1} biochar (Chen et al., 2010; Cross and Sohi, 2011; Inyang et al., 2010; Kameyama et al., 2012; Lee et al., 2013; Yao et al., 2012). Further details on literature and assumptions used to select a pyrolysis program aimed at producing a stable, high-C biochar can be found in **Appendix C.**

2.2.2 Biochar characterization.

The pH of biochar and soil–biochar mixtures was determined in the laboratory by mixing 3 g of sample in 27 g of water (Lee et al., 2013), followed by 30 min of intermittent shaking, 30 min of settling, and measurement with a handheld meter (HI 98121, Hanna Instruments, USA). Total C and total N contents were determined on a CHN analyzer (628 Series, LECO Corp., St. Joseph, MI).

In the carbohydrate analysis, raw filtercake and filtercake biochar samples were finely ground and extracted in hot acetone for 12 h to remove extractives (fats, resins, etc.). Next, 0.2 g of the dried extracted sample were incubated for 2 h in 3 mL of 72% H_2SO_4 (at 20°C), and then diluted to a final concentration of 4% H_2SO_4 and autoclaved at 121°C for 1 hour, followed by filtration. The filtered hydrolysate was then analyzed for carbohydrates via high-performance liquid chromatography, as described in detail by Huntley et al. (2003).

Total and micropore surface area for both the initial feedstock (raw filtercake) and filtercake biochar were determined using the Brunauer–Emmett–Teller (BET) method (Brunauer et al., 1938), calculated from the N_2 adsorption isotherm captured using an ASAP 2020 Physisorption analyzer (Micromeritics, Norcross, GA). Before analysis, samples were degassed at 90°C for 1 hour under a vacuum. For scanning electron microscopy (SEM), samples were sputter-coated with gold and analyzed using a Shimadzu SSX-550 microscope with an accelerating voltage of 15 kV. Finally, insight into the molecular structure of the biochar samples was gained through Raman spectroscopy, performed using a LabRAM HR system (HORIBA Jobin Yvon S.A.S., France). Spectra were captured using a 442-nm laser source, with a laser power on the sample

surface of 1 mW and a 100× objective lens. Samples were acquired over an integration time of 60 s with two scans per sample. Data were analyzed using LabSpec 5 software (HORIBA Jobin Yvon S.A.S., France).

2.2.3 Nutrient availability in soil–biochar mixtures

Changes in macro- and micronutrient availability were analyzed using soil–biochar mixtures made up of the field soil with increasing amounts of biochar (0, 1.25, 2.5, or 5% biochar on a dry weight basis). The mixtures were analyzed in solutions prepared at 1:2.5 ratios (soil–biochar mixtures:distilled water). Analyses were carried out according to the standard soil methodologies published by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, 2009). These included the following determinations: available P, Mehlich I extraction with spectrophotometric determination; K^+ and Na^+, Mehlich I extraction with flame photometry; Zn, Cu, Mn, diethylenetriaminepentaacetic (DTPA) extraction followed by atomic absorption; Fe, Mehlich I extraction with atomic absorption; Ca^{2+} and Mg^{2+}, KCl (0.1 M) extraction followed by the complexometric titration method (EDTA method); Al^{3+}, KCl (0.1 M) extraction followed by titration with NaOH; S, Ca_2PO_4 extraction followed by spectrophotometric determination; B, hot water extraction followed by colorimetric determination. Cation exchange capacity (CEC) was calculated as the sum of exchangeable bases (Ca^{2+}, Mg^{2+}, and K^+) plus potential acidity (H^+ + Al^{3+}).

2.2.4 Water retention in soil-biochar mixtures

The effect of biochar on water retention was analyzed using the same soil–biochar mixtures described in **Section 2.2.3**. The water potential of these mixtures was analyzed using a WP4C

Dewpoint Potentiameter (Decagon Devices Inc., Pullman, WA). Briefly, each of the oven-dried soil–biochar treatments (n = 3 for each treatment) were divided into 6 sub-samples which were then moistened with 0, 0.5, 1.0, 1.5, 2.0, or 3.0 mL of ultrapure water. These were allowed to equilibrate for at least 16 h, and then ~0.5 g of each sub-sample were analyzed for soil water potential. Immediately after, each sub-sample was weighed, dried at 105°C overnight, and weighed again to determine soil water content. To facilitate the statistical analysis of replicates, water potential values (in pF) were plotted against binned soil water contents.

2.2.5 CO_2 efflux assay

The mineralization of raw *vs.* pyrolyzed filtercake in the presence of absence of vinasse was analyzed in the laboratory. Treatments were established as follows: soil alone (S); soil + 5% filtercake (SF); soil + 5% biochar (SB). Raw filtercake and biochar used to prepare these treatments were both sieved to 2 mm. To minimize disturbance, fresh, field-moist soil was sieved to 4 mm, roots were removed by hand, and then the soil was gently mixed to ensure a uniform composition. Treatments (biochar or raw filtercake sieved to < 2 mm) were then mixed into the field-moist soil. Equal weights of these treatments were packed into incubation columns (10 × 15 cm, D × L; n = 6 each) and left open to the atmosphere in a temperature-monitored environment. After a 24-h equilibration period, CO_2 efflux was determined on a daily basis for three weeks using a LI-COR 6400 XT apparatus (LI-COR, Lincoln, NE), which was fitted over the incubation column using a PVC sleeve and a foam rubber gasket. CO_2 measurements were collected over three weeks under three conditions: week 1, field-moist (no added water); week 2, 30 mL water or vinasse (low dose); week 3, 60 mL water or vinasse (high dose). Thus, in total, six soil–amendment treatments were created: S, soil alone; SV, soil with vinasse; SF, soil with

raw filtercake, SVF, soil with filtercake and vinasse; SB, soil with biochar; SVB soil with biochar and vinasse. Vinasse and water were applied by slowly and uniformly dripping liquid over the soil surface using a pipette. CO_2 fluxes were monitored over time and total CO_2 flux and % C released was determined. Incubations were carried out at ambient temperature, ranging from 25–29°C during the night and 29–35°C during the day.

2.2.6 Statistical analyses

Treatment effects from soil–biochar mixtures (**Section 2.2.3)** and soil–filtercake/biochar/vinasse mixtures (**Section 2.2.5)** were analyzed using one-way analysis of variance (ANOVA) with *post hoc* Tukey tests to detect significant differences among means. All data are presented as the mean ± the standard error of the mean (SE). A p-value < 0.05 was considered significant. All statistical analyses were carried out in R v.3.0.1 (R Core Team, 2013) using Rcmdr (Fox, 2005).

2.3 Results

2.3.1 Filtercake biochar production and characterization

The pyrolysis of raw filtercake yielded what appeared to be a fully pyrolyzed product upon visual inspection (*i.e.,* uniformly black, very friable), as well as an unquantified amount of bio-oil. Biochar yield was equal to 36% of the dry feedstock weight. Filtercake biochar was alkaline in water (pH = 9.85 ± 0.08) and contained 36.7% C dry weight and 1.3% N dry weight (C:N, 28), whereas raw filtercake contained 37.4% C and 1.2% N by weight (C:N, 32). Pyrolysis greatly depleted but did not wholly eliminate carbohydrates. Raw filtercake contained 18% cellulose (d.w.) and 11.9% hemicellulose, as well as 32% lignin; filtercake biochar retained 2.7% cellulose and 3.1% hemicellulose. The lignin content of biochar was not determined because the

protocol used here (the Klason method), is unable to distinguish between lignin and the non-acid-soluble aromatic C structure of the biochar itself.

The biochar was analyzed by Raman spectroscopy, which revealed a characteristic two-peak spectrum (**Fig. 2.1**) typically displayed by carbonaceous materials with a core aromatic structure, including biochars (*e.g.*, Fuertes et al., 2010). The first peak, originally designated the D or *defect* band (1360 cm^{-1}), was initially associated with disordered graphite structure, but has since been associated with benzene rings (Kim et al., 2011). The G or *graphite* band (1590 cm^{-1}) is characteristic of graphite; however, in the case of chars, previous work showing the lack of detectable graphitic C via X-ray diffraction led authors to attribute this peak instead to quadrant aromatic ring breathing (Asadullah et al., 2010). These results suggest that the filtercake biochar produced is primarily composed of aromatic C.

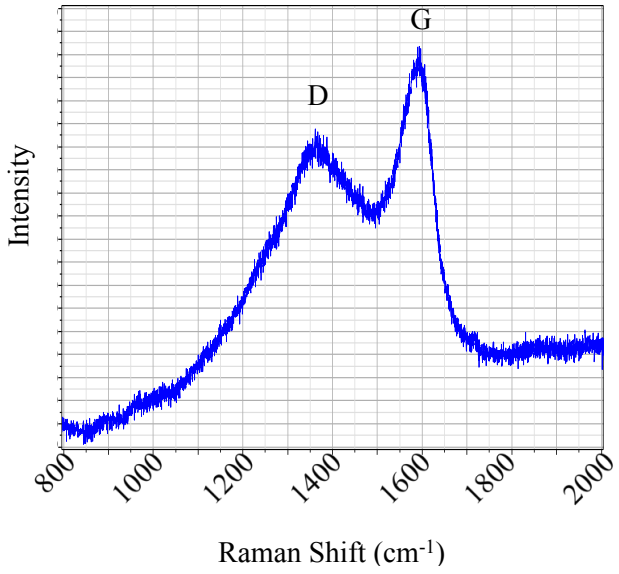

Figure 2.1. Raman spectroscopy.

Raman spectrograph of filtercake biochar showing characteristic features of charred aromatic material. D, defect band (1360 cm^{-1}); G, graphite band (1590 cm^{-1}).

Finally, N_2-BET surface analysis revealed that slow pyrolysis increased the specific surface area of raw filtercake from 0.38 $m^2\ g^{-1}$ to 26.3 $m^2\ g^{-1}$ in the charred product. Analysis of deBoer t-plot micropore area revealed that only approximately 5.5 $m^2\ g^{-1}$ of this surface area was contained within pores < 2 nm in diameter. Scanning electron microscopy of both the raw filtercake and pyrolyzed product revealed differences in particle size due to fracturing during pyrolysis (**Fig. 2.2-A, 2.2-B**). Biochar particles were angular with large irregular macropores. At high magnification (2400×), some biochar particles showed scant formation of pores < 1 μm in diameter (**Fig. 2.2-C**).

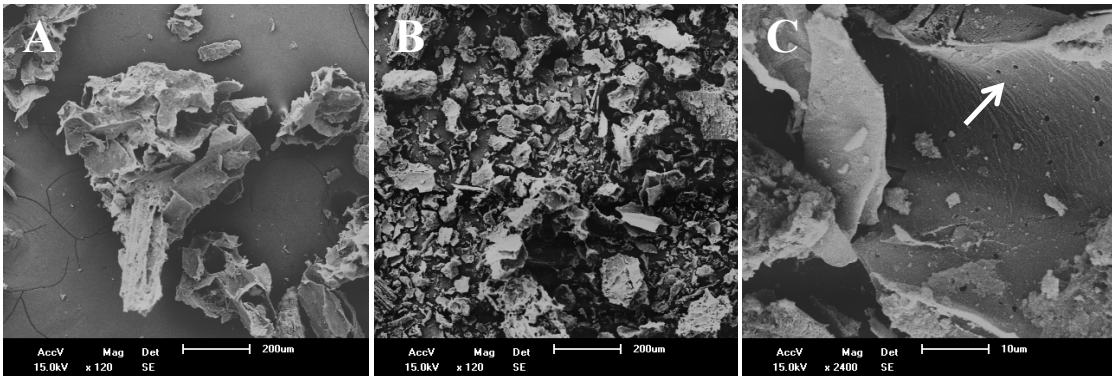

Figure 2.2. Scanning electron micrographs of filtercake and filtercake biochar.

(A) Raw sugarcane filtercake at a magnification of 120×. (B,C) Filtercake biochar at magnifications of 120× and 2400×, respectively. White arrow indicates micropore formation.

2.3.2 Effect of biochar on soil quality parameters and nutrient extractability

Mixing an air-dried dystrophic red-yellow Ferralsol with increasing amounts of filtercake biochar led to increases in soil pH, cation exchange capacity, and C, nitrogen, and nutrient availability for increasing levels of biochar (**Table 2.1**). Even the low dose of 1.25% biochar

markedly increased the availability of P, K, and Ca. Among micronutrients, the addition of biochar most strongly affected the extractability of Fe, Mn, and Zn, although these effects were only apparent at a treatment of 5%. This increase in extractable nutrients may be due to a direct contribution from the biochar amendment, as well as increases in nutrient availability due to the pH change resulting from the addition of biochar.

	---------- % Biochar (by weight) ----------			
Parameters	***0%***	***1.25%***	***2.5%***	***5%***
pH (H_2O)	6.13 ± 0.03, d	6.70 ± 0.06, c	7.03 ± 0.03, b	7.40 ± 0.06, a
CEC	8.0 ± 0.2, b	9.0 ± 0.2, b	10.5 ± 0.2, a	11.6 ± 0.4, a
% C	1.48 ± 0.06, b	--	--	2.65 ± 0.03, a
% N	0.07 ± 0.01, a	--	--	0.08 ± 0.01, a
Macronutrients				
P	2.5 ± 1.0, b	107.8 ± 5.8, a	146.0 ± 22.8, a	151.6 ± 17.8, a
K	46.7 ± 0.7, d	114.3 ± 3.2, c	164.3 ± 3.5, b	243.0 ± 10.8, a
Ca	3.1 ± 0.1, c	4.9 ± 0.1, b	6.1 ± 0.1, a	6.7 ± 0.3, a
Mg	1.5 ± 0.1, c	2.0 ± 0.1, c	2.7 ± 0.2, b	3.5 ± 0.2, a
S	13.1 ± 0.7, a	13.9 ± 0.1, a	13.1 ± 0.1, a	13.2 ± 0.3, a
Micronutrients				
B	0.26 ± 0.02, a	0.27 ± 0.02, a	0.29 ± 0.01, a	0.28 ± 0.01, a
Fe	115.0 ± 12.8, b	173.7 ± 14.5, ab	195.7 ± 18.8, ab	223.7 ± 29.6, a
Mn	7.1 ± 0.9, b	8.0 ± 0.9, b	10.1 ± 0.7, ab	12.9 ± 0.5, a
Zn	2.8 ± 0.6, b	3.7 ± 0.6, b	4.3 ± 0.2, b	6.4 ± 0.1, a

Table 2.1. Physicochemical characterization of soil–biochar mixtures.

Statistical comparisons were performed using one-way analysis of variance with a *post hoc* Tukey test. Letters in common indicate a non-significant difference between treatments. Data represent the mean ± SE. P and K values are given in mg dm^{-3}. Remaining nutrient values and CEC are given in cmolc dm^{-3}.

2.3.3 Water retention

The effect of biochar on soil water retention was examined by generating matric potential–soil moisture curves for soil alone and soil mixed with 5 or 10% biochar. The addition of 5 or 10%

biochar led to a dose-dependent increase in soil matric potential at a given soil moisture content; importantly, this effect was only observed within the range of soil water potentials coinciding with plant-available water (pF < 4.18), and grew more pronounced at the wetter end of the curve (**Fig. 2.3**). These data suggest that filtercake biochar increased the availability of pores that release water at potentials less than pF = 4.18 (pores > 0.2 μm in diameter) (Hamblin, 1985). This is consistent with our N_2-BET surface area analysis, which found that this filtercake biochar showed very little surface area in pores < 0.2 μm in diameter, which is desirable in terms of retaining plant-available water.

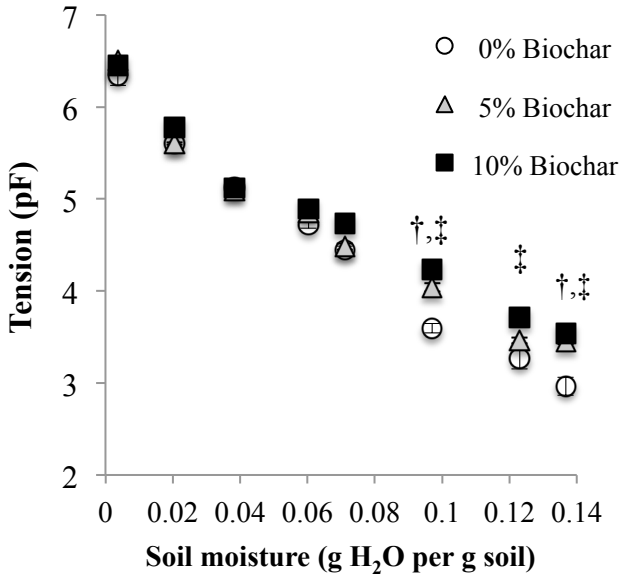

Figure 2.3. Soil matrix potential in relation to changes in moisture content.

Soil matrix potential was analyzed in relation to changes in moisture content in soils containing 0, 5, or 10% filtercake biochar. Treatments were compared using one-way ANOVA with a *post hoc* Tukey test. Data represent the mean ± SE. Note that significant differences were observed only below the permanent wilting point (dashed line). †, significant with respect to 5% biochar treatment; ‡, significant with respect to 10% biochar, $p = 0.05$.

2.3.4 Short-term CO_2 effluxes

To examine the effect of sugarcane residues on soil respiration and C utilization, field-moist soil was mixed with filtercake or biochar and incubated for three weeks. During the first 7 days, a large initial efflux of CO_2 was observed for soil amended with raw filtercake (SF), which peaked after two days; the soil plus biochar (SB) and soil-only treatment showed a more muted response that began to decrease immediately **(Figure 2.4)**. Respiration was also moisture-limited, as evidenced by brief increases in CO_2 efflux that accompanied water or vinasse addition (gray arrows in **Fig. 2.4**). Among treatment groups, soil-only (S) incubations showed the lowest mean effluxes, whereas biochar incubations (SB) were slightly but consistently higher over time. Vinasse application increased CO_2 efflux in the vinasse-only (SV) and vinasse+biochar (SVB) incubations relative to the S and SB controls, respectively. After calculating total g CO_2 emissions over the entire experimental period, we found that the addition of raw filtercake to soil (SF) led to a 100-fold increase in g CO_2 emitted relative to the unamended soil (S) control. However, this large increase was greatly ameliorated by applying the filtercake as a pyrolyzed product (**Table 2.2**).

Treatments	*CO_2 emitted ($g\ CO_2$)*	*Initial C content (g)*	*Initial C lost (%)*
S	0.24 ± 0.01, a	18.3	0.35 ± 0.01, a
SV	0.47 ± 0.01, a	18.4	0.70 ± 0.01, b
SVB	1.17 ± 0.13, b	40.7	0.78 ± 0.08, b
SB	1.04 ± 0.03, b	40.6	0.69 ± 0.02, b
SF	23.9 ± 0.55	40.1	16.29 ± 0.37
SVF	23.9 ± 0.36	40.3	16.25 ± 0.24

Table 2.2. Mass (g) CO_2 emitted and percent initial carbon lost from cultivated soil.

To further probe differences among the S, SV, SVB, and SB treatments, total CO_2 emitted and % C lost over the 3-week experimental period were compared via one-way ANOVA. Data represent the mean ± SE. Similar letters indicate a non-significant difference between treatments as determined using the Tukey HSD test. Because filtercake addition greatly skewed the data set, SF and SVF treatments were analyzed independently using a two-tailed unpaired *t*-test and found not to differ significantly.

To further investigate the effects of biochar and vinasse on soil C emissions, data were analyzed with respect to percent C lost from the treatment, based on a calculated estimate of the amount of soil C plus amendment C added (**Table 2.2)**. Because the powerful effect of raw filtercake addition greatly skewed the data set, we examined the differences among the S, SV, SVB, and SB treatments separately using one-way ANOVA. This revealed that although total C loss from soil-only incubations was low (S, 0.35 ± 0.02% of total C present), vinasse addition did significantly increase overall % C lost (SV, 0.70 ± 0.02%, $p = 0.003$), as would be expected given the high bioavailability of this residue. Biochar addition alone (SB) also led to a minor increase in the total C lost (0.69 ± 0.15%, $p = 0.003$ with respect to S), perhaps due to increased aeration as a result of a slight decrease in soil bulk density (S, 1.08 g cm^{-3}; SB, 1.04 g cm^{-3}, $p < 0.001$). Vinasse addition did not augment this effect (SVB, 0.78 ± 0.04%) compared to biochar alone (SB, 0.69 ± 0.15%, $p = 0.54$). In contrast, filtercake treatments lost a much greater

percentage of total C (SF, 16.3 ± 0.4% C), most likely due to a much larger proportion of filtercake feedstock that was enriched in cellulose and hemicellulose (approx. 55% of total C). Vinasse application with filtercake had no additional effect (SVF 16.2 ± 0.2%), perhaps because the soil had reached its respiratory maximum for the available amount of water.

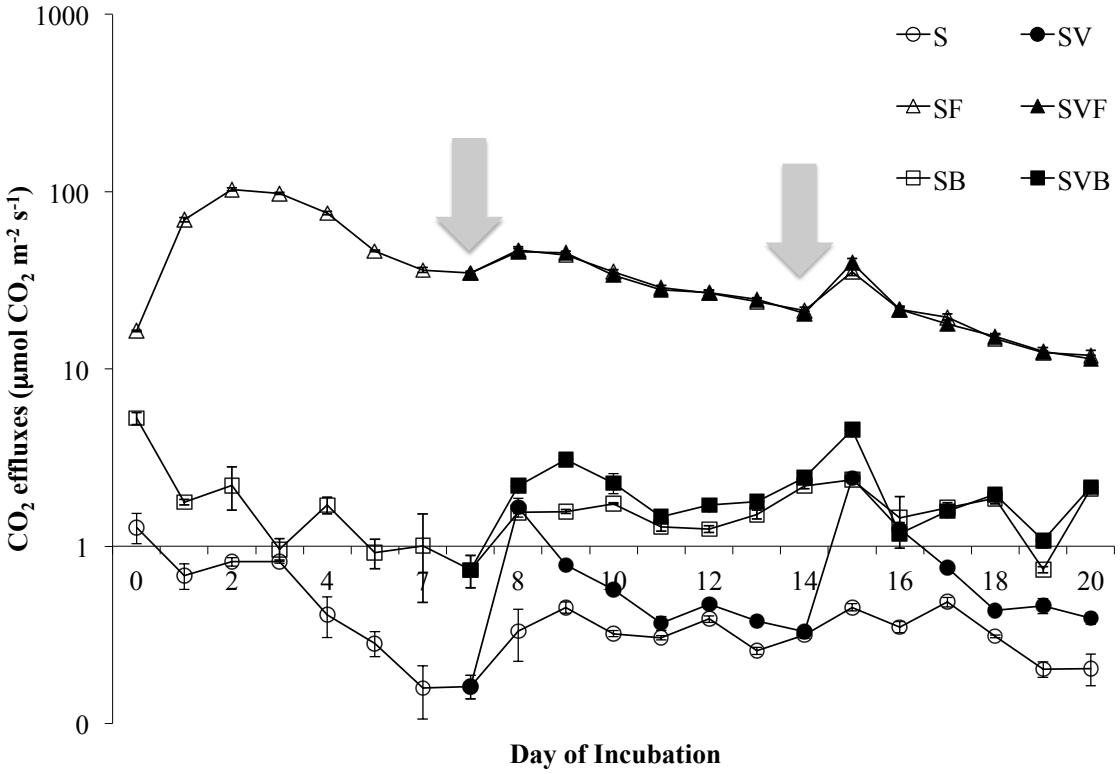

Figure 2.4. Daily CO_2 effluxes from a residue-amended cultivated soil.

Cultivated soil was amended with 5% filtercake or 5% biochar. Data points represent the mean treatment value ± SE at each time point. Note that untransformed data are plotted here on a logarithmic scale to facilitate viewing. Water (open symbols) or vinasse (solid symbols) was added on the days indicated by a gray arrow; 30 mL were added at the beginning of week 2 and 60 mL at the beginning of week 3. S, soil; SV, soil with vinasse; SF, soil with 5% filtercake (d.w.); SVF, soil with 5% filtercake and vinasse; SVB, soil with 5% biochar and vinasse; SB, soil with 5% biochar.

2.4 Discussion

It is frequently noted in the biochar literature that feedstock and pyrolysis conditions are the determining factors in final char characteristics (Cross and Sohi, 2011; Joseph et al., 2009; Kameyama et al., 2012; Lee et al., 2013). Here, we compare filtercake biochar with its related product, bagasse biochar as reported in the literature, as well as other feedstocks, and compare these in terms of their agronomic benefits.

2.4.1 Physicochemical characterization of filtercake biochar

In this study, filtercake biochar produced under the described pyrolysis conditions falls under the category of a low-C char, according to the biochar classification proposed by Joseph *et al*. (2009). This is somewhat surprising given that our feedstock was relatively rich in lignin (32%) compared to softwoods and other non-woody residues (Arsene et al., 2013). Previous work has shown that lignin has a higher char yield (C in feedstock retained in char, 49%) compared to cellulose (19%) or hemicellulose (23.5%) after pyrolysis at 800°C (Cagnon et al., 2009), and feedstocks rich in lignin often produce chars with higher % C (Demirbas, 2004; Lee et al., 2013). For example, bagasse with an initial lignin content of approximately 20% (Arsene et al., 2013; George et al., 2010), produces chars in the range of 63–84% C at 600°C (Chen et al., 2010; Inyang et al., 2010; Kameyama et al., 2012). Thus, the filtercake char described here had less C than might be expected for a lignin-rich material pyrolyzed at close to 600°C. Because filtercake has been subjected to fermentation, the depletion of biodegradable non-lignin C may account for both the overall low C yield in the biochar product as well as lignin enrichment in the filtercake. Nevertheless, filtercake biochar may be very valuable as an agricultural amendment. Regarding its effects on nutrients, treatments as a low as 1.25% biochar (d.w.) significantly increased P, K,

and Ca availability and treatment at 5% increased micronutrient availability (**Table 2.2**) in a dystrophic red-yellow Ferralsol. Furthermore, in an agricultural context, the presence of a small amount of non-pyrolyzed carbohydrates can be viewed as a benefit as these compounds represent a source of bioavailable nutrients and energy for the soil microbial community.

Surface area is a key physical characteristic of biochars because it indirectly indicates a char's ability to retain water as well as dissolved nutrients and low-molecular-weight C compounds through pore filling (Kasozi et al., 2010). In this study, we noted that filtercake biochar pyrolyzed at 575°C had a high N_2-BET surface area (26 m^2 g^{-1}) compared to the raw filtercake feedstock (0.38 m^2 g^{-1}), but a relatively low N_2-BET surface area compared to bagasse pyrolyzed at close to the same temperature (218 m^2 g^{-1} at 600°C) (Kameyama et al., 2012). Further SEM analyses revealed highly angular, macroporous particles (**Fig. 2.2-B**), demonstrating why biochar addition decreased bulk density and increased porosity in treated soils. Pore formation depends largely on production parameters and original biomass structure (Brewer et al., 2009). Although some remnants of the original plant vasculature were observed through SEM (data not shown), filtercake biochar overall lacked the regular, highly porous structure observed in bagasse biochar made at near the same temperature (Inyang et al., 2010; Lee et al., 2013). Because filtercake is subject to maceration and fermentation, destruction of the plant's vascular structure may have contributed to the relatively low total surface area.

Nevertheless, even this small increase in total surface area and porosity over the raw feedstock may underlie the increase in soil matrix potential in soil–biochar mixtures. Our results showed that biochar dose-dependently increased the soil matric potential for a given quantity of water

added, indicating that water was held more tightly, and this effect was significant within the range of plant-available water (pF < 4.18). This work is consistent with numerous previous studies showing that biochar increases plant-available water, especially in sandy soils similar to the one examined here (Abel et al., 2013; Basso et al., 2013; Kameyama et al., 2012; Ulyett et al., 2014). Given that modeling studies have predicted a warmer, drier future for this region of Brazil (Hoffmann and Jackson, 2000), utilizing filtercake as biochar to increase plant-available water may be a useful climate adaptation strategy.

2.4.2 Bioavailability of filtercake biochar *vs.* raw filtercake

Biochar is also posited as a possible climate mitigation strategy, based on the assumption that aromatic C within biochar is highly resistant to microbial attack, and may therefore increase C sequestration (Mathews, 2008). However, the effect of biochar on soil respiration and C turnover is complex. Although some studies have reported that biochar suppresses or has no effect on soil respiration (Cross and Sohi, 2011; Spokas and Reicosky, 2009), others report a transient or sustained increase in CO_2 efflux (Cross and Sohi, 2011) and microbial biomass (Steinbeiss et al., 2009) over the study periods, suggesting a biological response. We also noted a small but sustained increase in CO_2 efflux from biochar-amended soils relative to the untreated control, and that biochar-treated soils lost a minor, though overall greater percentage of total C compared to the unamended soil (**Table 2.2**). This may indicate a priming effect, which is defined as an increase in the turnover of existing SOC in response to C addition that is mediated by soil microbes (Kuzyakov, 2010); these priming effects occur in many systems and their ecological consequences are not well understood. Alternatively, increased CO_2 efflux in the presence of biochar may occur through an abiotic mechanism, such as oxidation or off-gassing of CO_2,

which in some studies have accounted for >50% of CO_2 emitted from heat-sterilized biochar alone (Jones et al., 2011) or sterile sand–biochar mixtures (Zimmerman, 2010). Regardless, the amount of C lost via abiotic or biotic mechanisms seems to be dependent on biochar production temperature; chars produced at higher temperature with lower remnant labile or volatile C produced a smaller or suppressed efflux (Zimmerman et al., 2011). Thus, although the filtercake biochar used here was produced at a relatively high temperature, the residual cellulose and hemicellulose detected in the carbohydrate analysis may have been responsible for elevated soil CO_2 effluxes.

2.4.3 Use of raw *vs.* pyrolyzed filtercake in sugarcane cultivation

Recent lifecycle assessments examining the contribution of SOC to total C emissions from biofuel systems have generated new appreciation for crop residue management and its role in keeping C in the soil (Liska et al., 2014). Returning sugarcane residues to the field is promoted as a means to re-build C stocks and recapture valuable nutrients (Anderson-Teixeira et al., 2009; Elsayed et al., 2008; Prado et al., 2013). However, residue co-application with mineral fertilizer also provokes transient increases in N_2O and CO_2 emissions compared to fertilizer treatment alone (Carmo et al., 2013; Oliveira et al., 2013), suggesting that this biomass undergoes relatively rapid mineralization and may contribute to further negative effects through GHG emissions.[5] Perhaps because of this, sugarcane cultivation areas in Brazil often show low to no recovery of soil C relative to the pre-cultivated baseline, despite large annual inputs from these residues (Oliveira et al., 2010; Resende et al., 2006; Vasconcelos et al., 2010).

[5] In the studies mentioned here, increased N_2O emissions were attributed to both the additional organic and inorganic nitrogen present in the vinasse, as well as wetting of the soil surface and the creation of anaerobic conditions that favour denitrification.

To mitigate SOC degradation, several previous authors have called for the integration of biochar science into biofuel cultivation and crop management (Abiven et al., 2014; Mathews, 2008). The objective of this chapter was to reconsider the waste product, filtercake, and gauge its potential merits as a biochar soil amendment through chemical characterization and a panel of assays assessing the most critical agronomic benefits of a biochar. Filtercake biochar showed benefits in terms of increasing soil pH, CEC, nutrient availability, and water retention, compared to an unamended soil. These parameters are directly relevant to exploratory studies investigating the effect of biochar amendment on sugarcane productivity via the use of biophysical models (*e.g.,* APSIM-sugarcane (McCown et al., 1996)). Furthermore, raw filtercake led to a large increase in soil respiration and a greater percentage of initial C lost compared to biochar treatment, likely due to the immediate utilization of labile sugars present in the filtercake. Although this was a very short-term study, from which it is not possible to extrapolate long-term biochar stability, it demonstrates the large difference in biodegradability between filtercake in its raw *vs.* pyrolyzed forms.

Due to this rapid decomposition loss, the use of raw filtercake may represent a lost opportunity for soil improvement in this region, where a hot, semi-humid climate and nutrient-poor, leachable, acidic soils severely limit plant productivity and increase management costs (Goedert, 1983). In contrast, applying filtercake as biochar may slow the decomposition loss of organic C from the field compared to raw filtercake amendment, and may increase alkalinity, CEC, water retention, and nutrient availability relative to unamended soil. Biochar implementation may also have economic benefits, as high-temperature pyrolysis is exothermic and self-sustaining through

the production of syn-gas; it also generates saleable bioenergy products (bio-oil and biochar itself) and shows potential as a tradable GHG emission-reducing soil amendment (Gaunt and Lehmann, 2008). However, further studies are required to determine the long-term stability of this biochar and how this product might influence lifecycle C emissions, as well as its effects on plant productivity and the feasibility of incorporating pyrolysis bioenergy and biochar into plant operations.

2.4.4 Summary

Physical and chemical characterization revealed that the novel filtercake biochar described here has significant benefits in terms of increased CEC, pH, nutrient availability, and water retention relative to unamended soil. Importantly, we also found that filtercake applied as biochar greatly reduced soil CO_2 effluxes compared to the application of raw filtercake, without wholly eliminating microbial activity. These findings open the way to further studies examining the use and stability of filtercake biochar in the field, and demonstrate in principle how organic waste management on sugarcane plantations could be modified to improve soil quality and reduce CO_2 effluxes from cultivated areas.

Chapter 3: Biochar reduces DOC but not nitrate leaching in relation to vinasse application in a tropical sugarcane soil

3.1 Introduction

Brazil is currently the world's second largest producer of bioethanol, second to the United States (Renewable Fuels Association, 2013). When produced from sugarcane, bioethanol as fuel boasts the greatest net energy return on investment and the highest avoided emissions per cubic meter when substituted for gasoline (Von Blottnitz and Curran, 2007). However, sugarcane cultivation is associated with other environmental effects that are not included in energy assessments, such as impacts on water quality, including the effect of effluents on surface and groundwater (Martinelli and Filoso, 2008). Of particular concern is vinasse, an acidic, nutrient-dense liquid waste that is produced at a rate of approximately 12 L for every liter of ethanol. Due to its high eutrophication potential, vinasse is typically disposed of by diluting and applying to soils (*i.e.,* fertigation), which is cost-efficient and also serves to recapture waste nutrients and water (Prado et al., 2013).

However, despite regulation aimed at preventing water pollution due to vinasse disposal, inputs to ground and surface water from land-applied vinasse remain a challenge. Previously, Gloeden et al. (1991) showed that low to moderate vinasse application (100 and 300 m^3 ha^{-1}) greatly increased dissolved organic carbon (DOC) and total Kjeldhal nitrogen in the saturated zone of both treated and non-treated adjacent sugarcane fields, demonstrating the high vertical and lateral mobility of vinasse constituents. At the landscape scale, Gunkel et al. (2006) found that vinasse

application within the catchment coincided with sharp increases in biological and chemical oxygen demand, and concurrent decreases in pH and dissolved oxygen in streamwater draining the catchment.

One of the proposed means to reduce leaching risk of applied soil amendments is through the use of biochar (charcoal derived from the pyrolysis of waste biomass), which has been shown to increase the retention of both cations and anions in leached soils (Major et al., 2009). In both temperate and tropical regions, biochars from wood and agricultural wastes have been found to modify the soil environment through increases in surface area, porosity, cation exchange capacity and pH (Abel et al., 2013; Angst et al., 2013; Inyang et al., 2010; Kameyama et al., 2012). These changes have been linked to a host of benefits, including increased water retention (Abel et al., 2013; Basso et al., 2013; Ulyett et al., 2014), enhanced microbiological activity (Lehmann et al., 2011; Steiner et al., 2009), and increased nutrient availability and crop yields (Major et al., 2010b; Petter et al., 2012; Steiner et al., 2009). In general, biochar benefits are most marked in sandy soils that are easily leached (Abel et al., 2013; Jeffery et al., 2011). Notably, however, tropical Ferralsols are characterized by the formation of clay microaggregates ranging from 80 to 300 μm in size, causing them to behave hydrologically like sandier soils (Balbino et al., 2004; Van Wambeke, 1974). Therefore, regions dominated by Ferralsols may derive a water retention benefit from biochar application, even when soils are not classified as sandy.

In the Brazilian Cerrado, a region in which sugarcane cultivation is predicted to increase rapidly in coming years (Martinelli and Filoso, 2008), the predominance of highly weathered, highly

leachable Ferralsols limits productivity unless substantial and costly amendments are applied (Furley and Ratter, 1988; Goedert, 1983). Sugarcane cultivation also produces large quantities of solid waste, including the nutrient-dense waste known as filtercake. Although filtercake is typically disposed of via soil application and provides some benefit as a fertilizer (Prado et al., 2013), previous work in our laboratory showed that filtercake converted to biochar provided pH, nutrient, and water retention benefits, and drastically reduced soil CO_2 efflux compared to the application of raw filtercake alone (as described in **Chapter 2**). Biochar produced from filtercake also increased soil porosity and cation exchange capacity relative to unamended soil, suggesting that it may be further valuable as a means to prevent nutrient leaching losses.

In this study, we conducted a laboratory column experiment to investigate the potential of filtercake-derived biochar to ameliorate DOC and nitrate (NO_3^-) leaching from vinasse-treated soil. We also examined the chemical quality of DOC passing through the treated columns using a combination of fluorescence and absorbance spectroscopy. Our data revealed that biochar reduced DOC leaching from treated soil through retention of humic acids originally present within the soil, but did not affect other low-weight constituents or NO_3^- derived from vinasse.

3.2 Materials and methods

3.2.1 Collection of soil and organic amendments

Mineral soil was collected from a cultivated sugarcane field at Usina Pantanal in Jaciara, MT, Brazil (15°55'28.11"S, 55°13'38.94"W, elevation 690 m). This region has a mean annual temperature of 25.5°C and mean annual precipitation of 1495 mm from 2000–2013 (BDMEP, 2013a). Soil was collected at the end of the dry season from a single soil excavation pit in a

sugarcane field that had been harvested in the previous month (no active plant growth). In order to facilitate re-creation of the soil profile in laboratory columns, the soil was collected in separate 10-cm layers to a 40-cm depth; these layers were then air-dried and sieved to 2 mm. The soil was classified as a red-yellow Ferralsol in the FAO soil taxonomy with a sandy clay loam texture (64% sand, 9% silt, 27% clay). Soil carbon contents before and after amendment with biochar and leaching were determined on a Carbon/Hydrogen/Nitrogen Analyzer (628 Series, LECO Corp., St. Joseph, MI); soil total carbon (C) and nitrogen (N) contents were 1.5% and 0.07% dry weight (d.w.), respectively.

Filtercake biochar was produced at the Federal University of Mato Grosso specifically for this study, and is described in detail in **Chapter 2**. Briefly, filtercake from Usina Pantanal was dried at 45°C and slowly heated inside a muffle furnace to 575°C at a rate of 5–6°C min^{-1}, with the temperature held at 575°C for 3 h, followed by slow cooling to room temperature. During pyrolysis, the cylinder was continuously flushed with an N_2 purge (0.5–1 L min^{-1}) to ensure oxygen-free conditions. Vinasse was collected from the sugarcane production area close to the point of application and kept frozen at –20°C prior to application to the experimental soil columns. Vinasse total C concentration (1.29 g C L^{-1}) was determined via combustion of the lyophilized solid residue (CHN-1110 elemental analyzer, Carlo Erba Instruments, Italy) with C content then multiplied by total solids. Vinasse and biochar characteristics are reported in **Table 3.1**.

Parameter	*Cultivated Soil**	*Biochar**	*Vinasse*
pH	6.6 ± 0.3	9.9 ± 0.1	5.2 ± 0.1
ORP (mV)	155 ± 6	40 ± 3	132 ± 3
Total C (g kg^{-1})	14.8 ± 0.1	367 ± 0.1	1.29 ± 0.04 g L^{-1}
Total N (g kg^{-1})	0.7 ± 0.1	13.2 ± 0.0	0.08 ± 0.00 g L^{-1}
C:N	21	28	16
Surface area (m^2 g^{-1})	N.D.	26	N.D.

Table 3.1. Physicochemical characterization of biochar and vinasse.

*** Biochar pH and oxidation-reduction potential (ORP) were taken in water. N.D., not determined.**

3.2.2 Soil column preparation and treatments

Twelve soil columns were constructed out of PVC pipes (10 cm in diameter, 50 cm in length) that were capped at their lower ends. The end cap was fitted with a brass coupling to serve as a vacuum sample collection port. Soil was prevented from escaping by first placing a 250-mesh steel screen, followed by a 2.5-cm layer of acid-washed, muffled glass beads. Next, the sieved, dried soil was packed into the columns in 1-cm lifts, with uniform compaction and scarifying between lifts to promote hydraulic continuity and ensure consistent density within and among columns (Lewis and Sjöstrom, 2010).

Triplicate columns were prepared for each treatment: control soil (S) with no amendments, soil that would receive vinasse (SV), soil mixed with biochar (SB), and soil mixed with biochar that would receive vinasse (SVB). For the biochar columns, only the top layer (0–10 cm) was mixed with ground biochar (< 2 mm) to make up 5% of the dry weight of the soil in that layer.
The columns were then conditioned for four months with water (S and SB treatments) or vinasse plus water (SV and SVB treatments) applied at one-month intervals. All columns were also flushed with water until DOC and NO_3^- concentrations of the SV and SVB columns returned to

baseline (determined in comparison to S and SB columns), and were then allowed to dry. Conditioning served to simulate rainfall and repetitive field applications of vinasse, as soils receiving vinasse applications may be fertigated several times during the harvest season. Following conditioning, the leaching experiment, which was carried out over a single day, ran as follows: (i) all columns were wetted with water (0.75 L); (ii) next, SV and SVB columns received vinasse (0.25 L) while S and SB received 0.25 L of water; (iii) all columns received an additional 2 L of water. In total, all columns received 3 L of water (S and SB) or water plus vinasse (SV and SVB). The total liquid applied to each column equates to an application depth of 382 mm, compared to an annual mean precipitation of 1495 mm y^{-1} for the study area (BDMEP, 2013a).

During the experiment, liquids were added slowly to the surface of individual columns using a 10-mL pipette to prevent ponding and sidewall flow. The base of each column was connected to a vacuum filtration unit (Millipore Corp., MA) containing a muffled 0.7-μm glass fibre filter over a 250-mL collection flask. The 12 filtration units were connected to a vacuum manifold to which a vacuum of −30 kPa (–0.3 bar) was applied; this tension is roughly equivalent to the water potential exerted at field capacity in a typical soil and is routinely used to sample soil columns (Singh and Hatton, 2010). Once 250 mL of sample was collected in the filtration flasks (which required approximately 1.25 h), the vacuum pump was briefly turned off while samples were transferred to glassware for analysis. The manifold allowed this to proceed without breaking the suction applied to the columns. Water quality analysis proceeded immediately upon sample collection. Overall, 12 samples were collected from each column over the course of an 18-h experiment.

3.2.3 Water quality analyses

Immediately after collection, the samples were analyzed using a UV-Vis spectrophotometer (Spectrolyser; S-can, Austria), which produces an absorption spectrum of the sample from 200−750 nm. Concentrations of NO_3^- and DOC were then determined from the absorbance spectrum of each sample using AnaPro software (S-can, Austria), which applies a global calibration for turbidity, TOC, DOC, and NO_3^- values determined via derivative spectroscopy based on many thousands of absorbance spectra (Broeke et al., 2006). Water quality parameters can be viewed continuously, which was used to monitor experimental progress. Importantly, we used the DOC rather than the TOC parameter to estimate C concentrations in these soil leachates, as previous work has shown that the TOC parameter is highly sensitive to changes in turbidity, whereas the DOC and NO_3^- parameters are not (Broeke, 2005). DOC values obtained using the spectrophotometer showed a strong relationship ($R^2 = 0.86$, $n = 9$) with DOC concentrations determined as non-purgeable organic carbon (NPOC) using a conventional TOC analyzer (Multi NC3100e; Analytik Jena AG, Germany). DOC data reported here have been corrected based on this relationship. In addition, specific conductivity was monitored in consecutive samples throughout the experiment using a handheld meter (HI 98129, Hanna Instruments, USA).

Fluorescence excitation-emission matrices (EEMs) were obtained for a sample set collected near the end of the experiment using an Aqualog spectrofluorometer (Horiba Scientific, NJ). Briefly, a 1.5-mL aliquot was analyzed in a quartz cuvette with an integration time of 1 s. The resulting data were first corrected for Raman scattering and inner filter effects using Origin8® software (OriginLab Corp., Northhampton, MA), and then normalized against the Raman signal of pure

water (Lawaetz and Stedmon, 2009). The intensity of peaks identified in the corrected and normalized EEMs were then analyzed for changes in maximum intensity across treatment groups, based on peak identifications previously observed in soil leachates (Fellman et al., 2010).

DOC quality indices were derived for the sample set using data obtained through both fluorescence and absorbance spectroscopy. These indices included the fluorescence index (Cory and McKnight, 2005; McKnight et al., 2001), specific UV absorbance at 254 nm ($SUVA_{254}$) (Weishaar et al., 2003), humification index (Ohno, 2002), freshness index (Wilson and Xenopoulos, 2008), and the E2:E3 ratio (Helms et al., 2008; Spencer et al., 2009). These indices are described in greater detail in the Results.

3.2.4 Statistical analyses

Treatment effects were analyzed using one-way analysis of variance (ANOVA) with *post hoc* Tukey tests to detect significant differences among means. All data are presented as means ± the standard error (SE). A p-value < 0.05 was considered significant. All statistical analyses were carried out in R using R Commander (Fox, 2005; R Core Team, 2013).

3.3 Results

3.3.1 DOC export and DOC quality in leachate

Biochar treatment significantly attenuated the total amount of DOC leached over the course of the experiment (**Fig. 3.1-A**), both in comparison to the soil control (10.27 ± 0.08 mg for SB *vs.* 12.95 ± 0.39 mg for S, $p = 0.004$), as well as in columns receiving vinasse (15.72 ± 0.36 mg for SVB *vs.* 19.10 ± 0.52 mg for SV, $p < 0.001$). When analyzing DOC leaching curves from which

total export was calculated (**Fig. 3.1-B**), we found that leachates from treatments containing biochar showed consistently lower DOC concentrations throughout the experiment. In the early water-only phase of the experiment, small increases in DOC concentrations were observed for the S and SV traces, which were likely the result of previous vinasse applications during conditioning (SV), as well as the slightly slower mobilization of soil C (S). In contrast, the SVB and SB traces declined consistently in the early phase. Approximately midway through the experiment, a sudden increase in conductivity (**Fig. 3.2**) indicated the breakthrough of freshly applied vinasse (conductivity of 1331 μS cm^{-1}) in the SV and SVB columns. During the late phase of the experiment, we likewise observed decreases in DOC concentrations in SVB (9.14 ± 0.38 mg L^{-1}) columns compared to SV columns (11.69 ± 0.87 mg L^{-1}, $p = 0.02$), as well as in SB columns (3.43 ± 0.02 mg L^{-1}) compared to S columns (4.57 ± 0.20 mg L^{-1}), although this last comparison did not reach statistical significance in the ANOVA ($p = 0.39$).

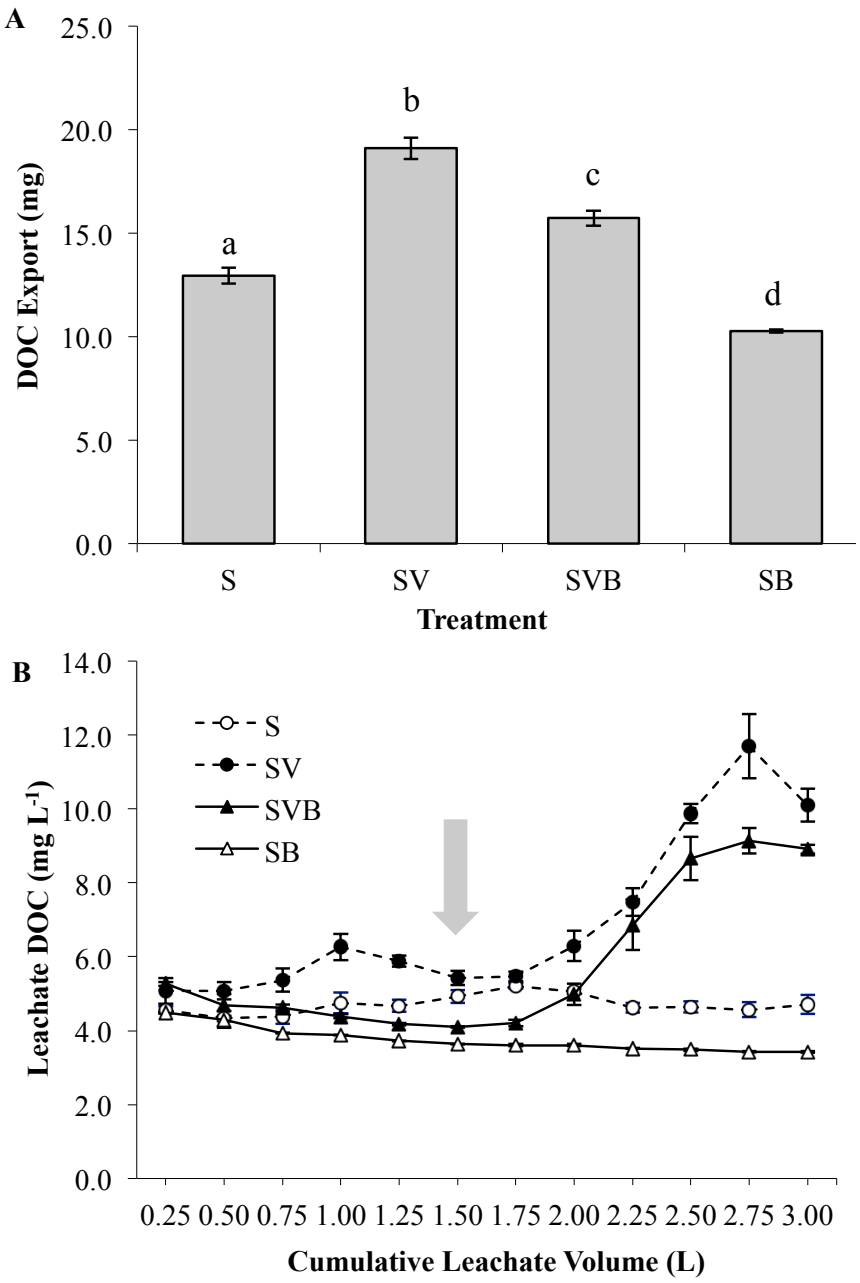

Figure 3.1. Effect of biochar on total DOC export and leaching curves.

(A) Total DOC export over the course of an 18-h experiment was estimated from the area under the breakthrough curves. (b) DOC breakthrough curves. Columns were leached with 0.75 L of water, followed by 0.25 L of vinasse (SV, SVB) or water (S, SB), and flushing with 2.0 L of water. Data represent the mean ± standard error (SE) for consecutive 250-mL samples (*n* = 3 for each treatment group). The gray arrow indicates vinasse breakthrough based on an increase in conductivity (Fig. 3.2).

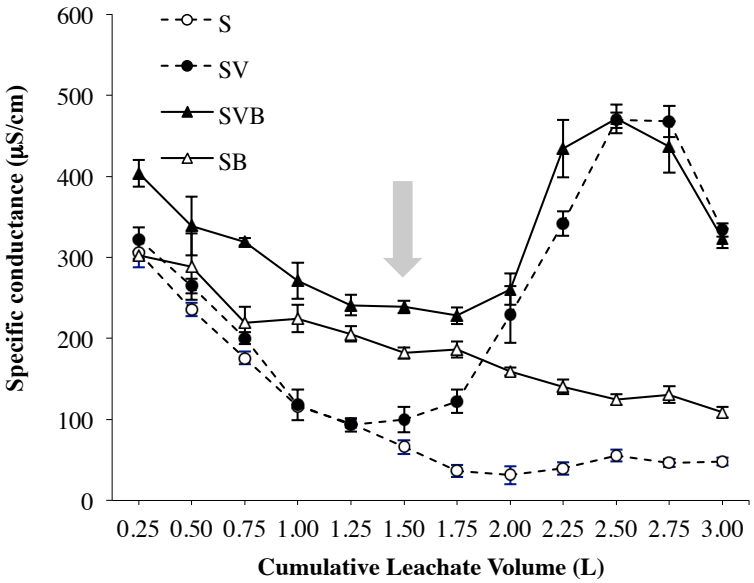

Figure 3.2. Changes in specific conductance during the leaching experiment.

Data represent the mean ± standard error (SE) for consecutive 250-mL samples (*n* = 3 for each treatment group). The gray arrow indicates vinasse breakthrough based on the observed sudden increase in conductivity in the SV treatment.

In addition to treatment differences in total DOC export (mg), biochar also altered the quality of DOC exported, as determined via absorbance and fluorescence spectrometry. In the EEMs derived from fluorescence analysis, the soil control (S) showed three weak peaks (**Fig. 3.3-A**), including a humic-like A peak (ex 240/em 435 nm), a tyrosine-like B peak (ex 285/em 297 nm), and a humic-like C peak (ex 334/em 406 nm), with a total fluorescence of 27.67 ± 1.47 RU (**Table 3.2**). Vinasse treatment greatly intensified peaks B and C and also introduced a fourth unknown peak at ex 240/em 300 nm for an increase in total fluorescence (53.60 ± 3.38 RU, $p < 0.001$; **Fig. 3.3-B**). However, when vinasse was applied over biochar-amended soil, the maximum intensities of peaks A and C were significantly attenuated (**Fig. 3.3-C**, **Table 3.2**), suggesting that biochar selectively retains humic substances, but not the relatively more mobile

amino acid-like compounds (peak B) contributed by vinasse. Total fluorescence was lower (SVB, 41.82 ± 1.82 RU) compared to SV columns ($p = 0.02$). The biochar-only control showed a marked decrease in total fluorescence (11.31 ± 0.14 RU, $p = 0.02$) and in the maximum intensities of peaks A, B, and C (**Fig. 3.3-D, Table 3.2**).

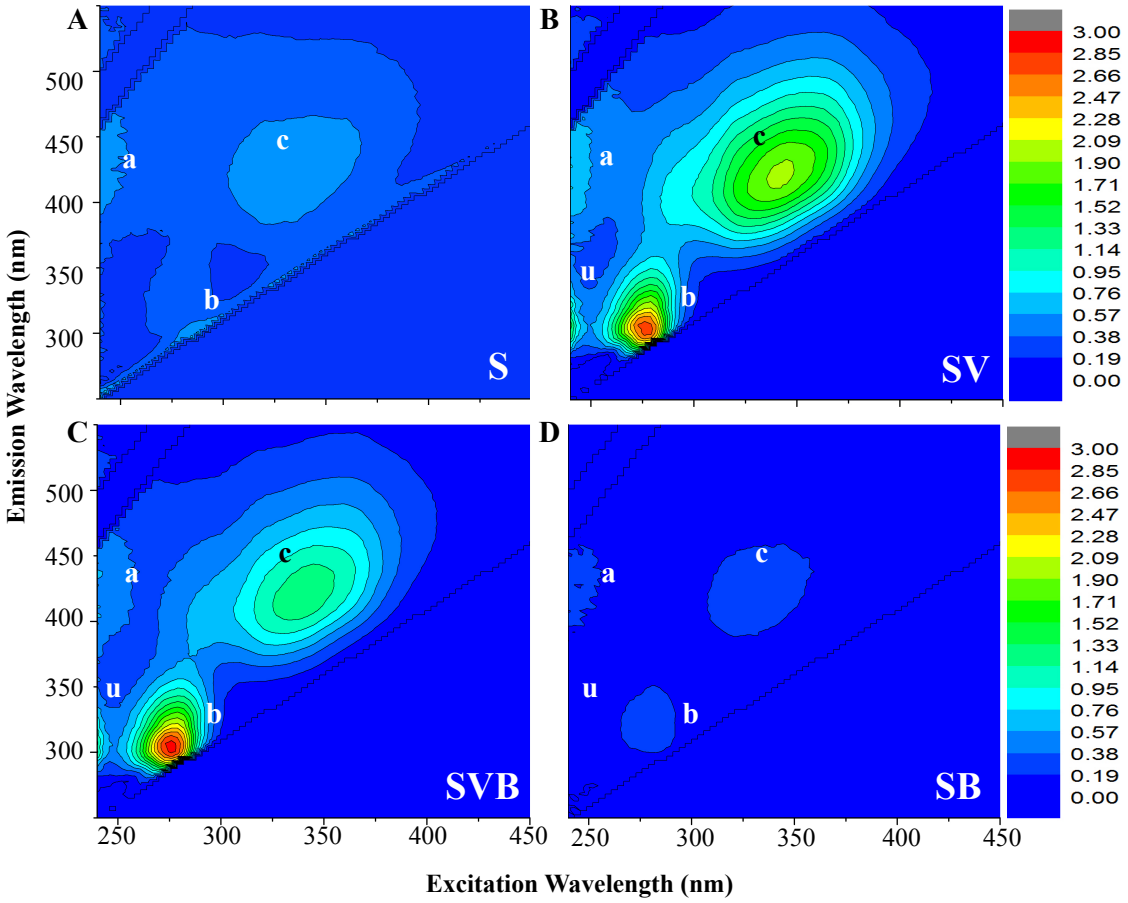

Figure 3.3. Representative excitation–emission matrices (EEMs) of leachates from each treatment group. Characteristic peaks have been labeled after Fellman et al. (2010): A (ex 240/em 424−454 nm), high-molecular-weight and aromatic dissolved organic matter from terrestrial (plant) sources; B (ex 276−285/ em 297−326 nm), amino acids free or bound in organic matter, from terrestrial or microbial sources; C (ex 324−345/em 336−425 nm), high-molecular-weight humic acids of terrestrial origin; U (ex 240/em 300−307 nm), unknown peak. The coloured bar represents fluorescence in Raman units (RU).

Indices	*Treatment*			
	S	***SV***	***SVB***	***SB***
Total Fluorescence (RU)	27.67 ± 1.47, *a*	53.60 ± 3.38, *b*	41.82 ± 1.82, *c*	11.31 ± 0.14, *d*
Peak Intensities (RU)				
A	0.63 ± 0.04, *ab*	0.76 ± 0.10, *a*	0.63 ± 0.02, *b*	0.29 ± 0.02, *c*
B*	0.78 ± 0.10, *a*	2.40 ± 0.18, *b*	2.60 ± 0.23, *b*	0.26 ± 0.03, *c*
C	0.63 ± 0.07, *a*	1.74 ± 0.13, *b*	1.29 ± 0.06, *c*	0.23 ± 0.01, *d*
Fluorescence index (FI)	1.63 ± 0.03, *a*	2.30 ± 0.02, *b*	2.29 ± 0.01, *b*	1.77 ± 0.00, *c*
Humification index (HIX)	0.71 ± 0.01, *a*	0.45 ± 0.01, *b*	0.35 ± 0.01, *c*	0.67 ± 0.02, *a*
$SUVA_{254}$ ($L\ m^{-1}\ mg^{-1}$)	3.25 ± 0.06, *a*	3.00 ± 0.00, *bc*	2.93 ± 0.01, *b*	3.10 ± 0.02, *c*
Freshness index (FrI)	0.71 ± 0.01, *a*	0.77 ± 0.00, *bc*	0.78 ± 0.01, *c*	0.74 ± 0.01, *ab*
E2:E3	1.92 ± 0.04, *a*	7.80 ± 0.76, *c*	8.92 ± 0.16, *c*	2.90 ± 0.11, *b*

Table 3.2. Fluorescence- and UV-Vis spectroscopy-based indicators of DOC quality.

All data are means ± SE (n = 3) and significance was set at $p < 0.05$. An asterisk indicates that data were log-transformed before analysis.

We used several optical indices to assess the effects of vinasse and biochar on DOC origin, humification, and molecular weight. These indices were determined for the sample set that exhibited peak DOC concentrations (*e.g.,* at 2.75 L total leachate collected, **Fig. 3.1-B**). Regarding DOC origin, the fluorescence index (FI) is derived from the EEM using the ratio of fluorescence emission intensities at 470 nm to 520 nm resulting from an excitation at 370 nm (McKnight et al., 2001). Lower FI values (~1.4) are observed for DOC for environments dominated by terrestrial sources of organic matter (plants and soil organic matter) and higher values (>1.9) are observed for DOC from environments heavily influenced by microbial processing and synthesis (Cory and McKnight, 2005). Vinasse caused significant increases in FI in both the SV and SVB treatments (**Table 3.2**), reflecting the strong presence of microbes and

the products of their metabolism in this effluent. This is consistent with our observation that peak B, a principally microbially-derived component, also did not differ between SV and SVB treatments. Furthermore, SB showed a slight increase in FI relative to the S treatment, which may indicate that the presence of biochar alone is sufficient to stimulate microbiological activity in the resting column. This is supported by previous studies showing that biochar addition increases soil CO_2 efflux (Cross and Sohi, 2011; Steinbeiss et al., 2009).

The degree of humification and complexity of leachate DOC was assessed using the indicators HIX, $SUVA_{254}$, and the freshness index (FrI). We found that HIX, which is directly proportional to the quantity of humified material in soil-extracted DOM (Ohno, 2002; Zsolnay et al., 1999) decreased markedly in SV columns (**Table 3.2)**. Similarly, the freshness index, which represents the contribution of recently produced to more decomposed or humified DOM (Fellman et al., 2010; Parlanti et al., 2000) increased in the SV and SVB leachates. $SUVA_{254}$, which is directly correlated with % aromaticity (Weishaar et al., 2003), also decreased in SV relative to S leachates. These results are consistent with the typical composition of vinasse, being primarily low-molecular-weight organic acids and sugars (Benke et al., 1998), which resulted in an overall shift to less complex DOC species in SV leachates.

Importantly, HIX and $SUVA_{254}$ decreased significantly again in the SVB treatment relative to the SV treatment, suggesting that biochar selectively retained higher molecular weight compounds, thus further increasing the proportion of less complex species in the leachate. SB leachates showed lower HIX and $SUVA_{254}$ values and higher FrI values relative to soil treatments, although the change in HIX and FrI were not significant at 0.05. These data suggest that biochar

preferentially retains humified DOC species derived from the soil itself, as well as from humic components present in vinasse. Finally, the E2:E3 ratio, which is inversely correlated with molecular weight (Helms et al., 2008; Spencer et al., 2009) showed increases for the SV, SVB, and SB treatments compared to the S treatment, providing further evidence that vinasse primarily contributes low-molecular-weight C and that biochar (alone or in combination with vinasse) selectively retains high-molecular-weight DOM and further increases the proportion of low-molecular-weight species in the leachate.

To complement our DOC leachate studies, we also analyzed total C in soil samples collected from each treatment group before and after leaching. Before leaching, biochar application (5% d.w.) resulted in a small but significant increase in total C (pre-SB, 2.65 ± 0.03%) compared to soil alone (pre-S, 1.48 ± 0.06%; $p < 0.001$). At the end of the experiment, at which point columns had been aged several months and subjected to several wet-dry cycles, total C levels remained significantly higher in the biochar treatments (SVB, 2.54 ± 0.53%; SB, 2.93 ± 0.28%) compared to the untreated columns (S, 1.37 ± 0.08%; SV, 1.42 ± 0.05%), indicating that the ground biochar remained in place in the 0–10 cm layer. In contrast, vinasse application (SV, 1.42 ± 0.05%) had no significant effect on total C compared to the untreated soil control before (pre-S, 1.48 ± 0.06%) or after (S, 1.37 ± 0.08%) leaching. These C data, as well as pre-and post-leaching data for pH, CEC, and macronutrients (P, K, Mg, and Ca) are further discussed in **Appendix E**.

3.3.2 Effects of organic residue treatments on NO_3^- leaching loss

Overall, biochar showed no effect on NO_3^- leaching loss in terms of total mg exported (**Fig. 3.3-A**). As expected, columns receiving vinasse showed increased NO_3^- export (SV, 6.99 ± 0.10 mg)

compared to soil-only columns (S, 5.07 ± 0.09 mg, $p = 0.003$). However, biochar amendment did not ameliorate NO_3^- loss (SVB, 7.56 ± 0.45, $p = 0.42$), but rather may have exacerbated leaching, as revealed through analysis of leachate breakthrough curves. In the early phase of the experiment, the presence of biochar had a marked effect on the shape of the NO_3^- release curve (**Fig. 3.3-B**), in which NO_3^- concentrations from biochar-treated columns (SVB and SB) peaked higher and earlier than in leachates from the S and SV.

After vinasse breakthrough (as indicated by an increase in conductivity, gray arrow in **Fig. 3.3-B**), a second rise in NO_3^- was again observed for the SV and SVB treatments. Notably, however, the amount of NO_3^- exported before vinasse breakthrough was much smaller than that released after breakthrough (**Fig. 3.2-B**), indicating that previous biological mineralization of organic N within the moistened column had a greater contribution on total export than did direct additions through vinasse treatment.

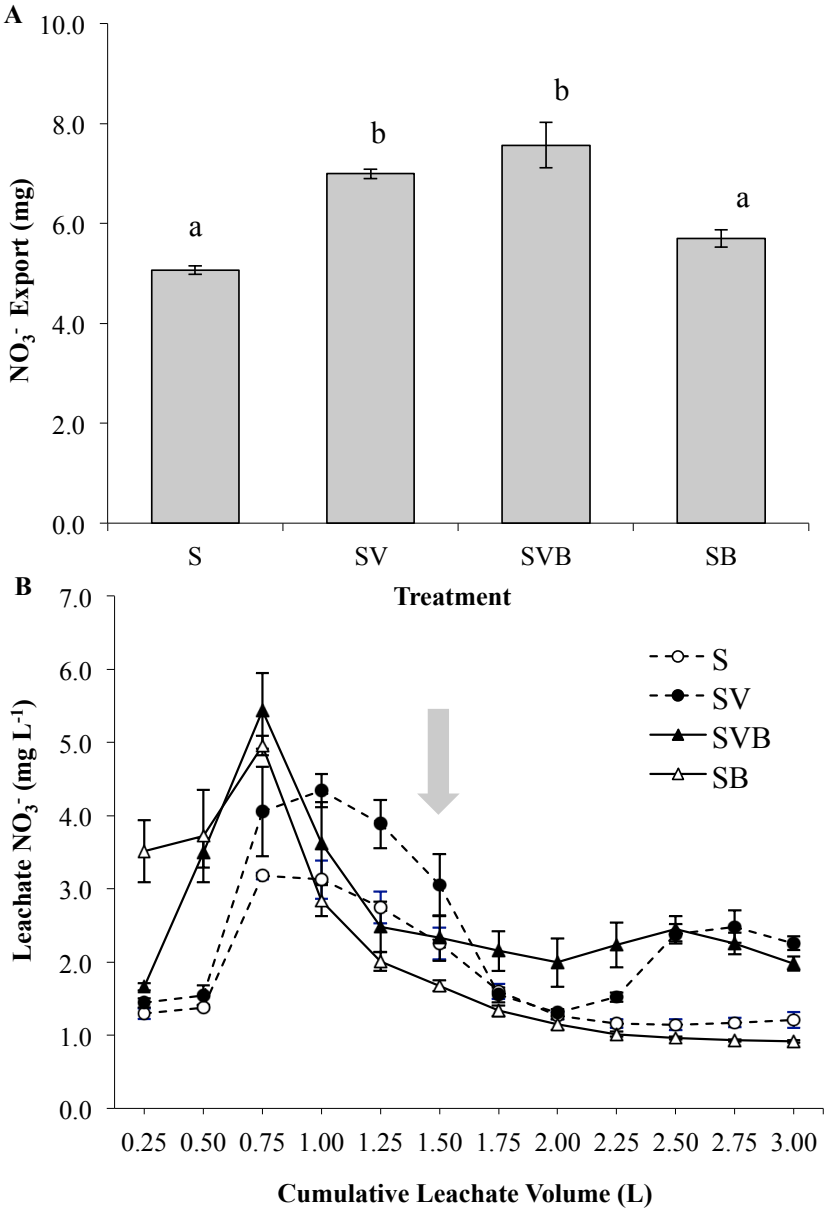

Figure 3.4. Effect of biochar on total (NO_3^-) export and leaching curves.

(A) Total NO_3^- export over the course of an 18-h experiment was estimated from the area under the breakthrough curves. (B) NO_3^- breakthrough curves. Columns were leached with 0.75 L of water, followed by 0.25 L of vinasse (SV, SVB) or water (S, SB), and flushing with 2 L of water. Data points represent the mean ± standard error (SE) for consecutive 250-mL samples collected over the course of the experiment (*n* = 3 for each treatment group). The gray arrow indicates vinasse breakthrough, based on increased conductivity (Fig. 3.2).

3.4 Discussion

3.4.1 Combined biochar and vinasse treatment may accelerate NO_3^- leaching loss

The effects of biochar on nitrogen cycling are complex due to the myriad effects that biochar addition has on the soil environment and its biota. Generally, biochars have a predominantly negative surface charge that grows more negative as they age in the soil (Cheng et al., 2008; Liang et al., 2006). However, it has been shown that increasing pyrolysis temperature (>600°C) for a given feedstock can result in a biochar that exhibits NO_3^--retaining rather than NO_3^--leaching properties (Kameyama et al., 2012; Yao et al., 2012). This may be due to a temperature-dependent shift in the ratio of basic to acidic surface functional groups that affects overall surface charge and may increase anion retention (Al-Wabel et al., 2013; Singh et al., 2010), particularly in fresh chars that have not yet been subjected to oxidation or ageing in the soil. The majority of recent studies reporting that biochar reduced NO_3^- leaching or enhanced soil accumulation have used fresh chars produced at >600°C, and were conducted over relatively short incubation periods (days to months) that may have precluded ageing effects (Angst et al., 2013; Knowles and Robinson, 2011; Novak et al., 2010; Yao et al., 2012; Zheng et al., 2013; Zwieten et al., 2010). Therefore, NO_3^--retaining chars may be an artifact of short-term studies rather than the norm over longer time scales.

In this study, the "aged" biochar in the SVB and SB columns had no attenuating effect on vinasse-derived NO_3^- additions, as demonstrated by similar total NO_3^- exports from SV and SVB columns. In fact, biochar inclusion seemed to potentiate NO_3^- release, as shown by the advanced peaking and rapid fall of the NO_3^- curves for SVB and SB in the early phase of the experiment (**Fig. 3.2-A**). This difference in rate of release may be due to observed changes in column water

pH (data not shown), which decreased as the experiment progressed. Because the point of zero net charge for red-yellow Ferralsols occurs at a pH of approximately 6.0, the columns may have shifted from NO_3^--releasing to NO_3^--retaining during the course of the experiment, which is supported by the relatively low NO_3^- release in the later half of the experiment. The non-significant trend toward increased total NO_3^- export in **Fig. 3.4-A** may also reflect the small but significant decrease in the density of biochar columns (SB, 1.33 ± 0.01 g cm^{-3}; S, 1.36 ± 0.00 g cm^{-3}, $p < 0.001$), resulting in more rapid flow. Previously, Kameyama et al. (2012) found that biochar inclusion significantly increased hydraulic conductivity, which would speed the loss of dissolved species. Changes in rate of release may also be due to increased negative charge density, as observed in soils with aged, naturally occurring black C (Liang et al., 2006) .

In addition to changes in the rate of NO_3^- loss, NO_3^- concentrations were higher overall in the early phase of the experiment, and biochar columns (SVB and SB) tended toward the highest NO_3^- values. This is consistent with other studies observing increased NO_3^- export from biochar-amended soils (Laird et al., 2010; Major et al., 2012; Singh and Hatton, 2010). Increased NO_3^- leaching may reflect a biochar-induced increase in the mineralization of soil organic nitrogen, perhaps due to increased oxygen availability in the more porous biochar-amended soils. It is well-documented that biochar addition may initially increase soil respiration, which then declines over weeks to years (Cross and Sohi, 2011; Major et al., 2010a; Steinbeiss et al., 2009), indicating heightened microbial activity that would also be expected to increase N mineralization in the presence of residues with low C:N ratios (here, C:N ratios were 25:1 for biochar and 16:1 for vinasse; **Table 3.1**). However, is also important to note that co-applying an organic

amendment alongside biochar may affect NO_3^- leaching loss as a result of pore-blocking that occurs in the presence of high DOC concentrations (Pignatello et al., 2006).

Overall, it is not possible to conclude that filtercake biochar has a positive or negative effect in terms of NO_3^-. Although a leaching loss was observed in soil columns here, the tendency toward increased NO_3^- availability in biochar-amended soils may increase plant productivity in a planted system (Major et al., 2012). However, it seems unlikely that this product can be used to mitigate NO_3^- leaching in situations where N from vinasse is applied greatly in excess of plant requirements.

3.4.2 Biochar and vinasse application and their effects on soil carbon

Biochar is widely proposed as a means to increase C storage by introducing a carbonaceous material that is chemically resistant to microbial attack, with variable results depending on char type and soil conditions (Zimmerman and Gao, 2013). Indeed, in this experiment, biochar addition resulted in a small but sustained increase in soil C content despite conditioning and leaching under warm, humid conditions for several months. However, a second means through which biochar may promote soil C sequestration is through stabilization of DOC that would otherwise be leached or quickly respired. In unamended soils, complex, aromatic C compounds are preferentially sorbed to the mineral component (Guo and Chorover, 2003), resulting in decreased mineralization of this sorbed organic matter to CO_2 (Kalbitz et al., 2005). Biochar may function in a similar manner. Although previous research has focused on biochar as a potential *source* of leachable C, this "first flush" is usually small relative to the amount of biochar C

applied (Major et al., 2010), and its magnitude varies greatly according to production technique and temperature, feedstock, and soil type (Lin et al., 2012).

Our study showed that the presence of "aged" biochar in the top 10 cm of a sandy soil led to a significant decrease in total DOC export during the experimental period. Similarly, Mukherjee and Zimmerman (2013) found that soil amendment with 1% (w/w) biochar produced at high temperature (650°C) decreased DOC leaching from a fine sandy Entisol in a laboratory leaching column experiment; however this effect was not observed in a similarly treated clay loam Ultisol. High-temperature chars also appear to have a higher sorption capacity for DOC than chars made at a lower temperature (Kasozi et al., 2010; Mukherjee and Zimmerman, 2013), which was attributed primarily to temperature-dependent variation in micropore surface area (Kasozi et al., 2010). These results suggest that high-temperature biochar might have its greatest effect on DOC leaching in coarsely textured soils where leaching is more likely to occur due to lack of clay minerals. However, given the strong microaggregate structure of clayey Ferralsols, and their subsequently altered hydrological properties that cause them to behave similarly to soils with much higher sand content (Van Wambeke, 1974), we may also expect to see biochar-induced benefits even in clay-rich soils.

In addition to a decrease in total DOC export, biochar inclusion also influenced the chemical quality of DOC leaving the column. Fluorescent peak analysis and spectroscopic indices suggest that biochar preferentially retained high-molecular-weight, more humified DOC species, whereas labile components were leached through. This may mean that biochar inclusion helps to retain more humified DOC already present in the soil rather than to capture DOC contributed by

vinasse that is mainly comprised of low-molecular-weight compounds (Benke et al., 1998). Indeed, the difference in mean DOC export between the S and SB treatments (2.7 mg DOC) was similar to that for the SV and SVB treatments (3.4 mg DOC). Furthermore, the co-application of vinasse and biochar (SVB) did not lead to an overall increase in soil % C compared to the SB treatment, as might be expected if biochar were able to stabilize vinasse DOC and protect it from leaching or rapid mineralization. These results indicate that although filtercake biochar decreased total DOC export, it likely did so through the stabilization of humified components already present within the soil. Retention of this native soil C may have occurred through the formation of organo-char complexes during the pre-experimental, ageing period (Cornelissen et al., 2005).

This presumed lack of vinasse DOC retention is surprising given that previous work on biochars produced from oak wood and Eastern gamma-grass showed sorption capacities for a low-molecular-weight compound (catechol) that were an order of magnitude greater than those for humic acid (Kasozi et al., 2010). These conflicting data may reflect differences in modes of adsorption that occur for different compounds. Kasozi et al. (2010) found that catechol adsorbed to high-temperature (650°C) biochar primarily through slow diffusion into micropores, which made up the greater part of total surface area; in contrast, humic acid adsorbed rapidly to the exterior of biochar particles through hydrophobic interactions, but total sorption capacity was much lower. The filtercake biochar produced in this study (at 550°C) had a N_2-BET surface area of 26 m^2 g^{-1}, which is 6- to 10-fold lower than the chars examined by Kasozi et al. (2010), and its area was mostly exterior surface rather than within micropores (< 0.2 μm in diameter). These differences, and the rapidity with which vinasse DOC was drawn through the columns, may have affected the relative ability of filtercake biochar to absorb low-molecular compounds enriched in

vinasse *vs.* the more complex compounds in vinasse (*i.e.*, a small amount of lignin) and in soil. Increasing pyrolysis temperature may also serve to increase micropore volume and increase adsorption capacity for hydrophilic low-molecular-weight compounds.

Finally, post-leaching soil analyses revealed that vinasse application did not significantly increase residual soil total C in SV columns in contrast to biochar-amended soil, which did maintain a significant increase in soil C content relative to the control. This is consistent with field studies demonstrating that vinasse application alone does not lead to sustained increases in SOC in sugarcane fields relative to the pre-cultivated baseline (Resende et al., 2006; Vasconcelos et al., 2010). This is likely due to the fact that sugarcane vinasse is primarily composed of low-molecular-weight sugars and organic acids (Benke et al., 1998) and is thus likely to be quickly metabolized in soil, or to leach as shown here. Thus, although vinasse does return C to the soil, its impacts on soil C appear to be short-lived.

3.4.3 Summary

This study provides new insight into the effects of filtercake biochar on the quantity and chemical quality of DOC leached from a cultivated soil after vinasse application. Furthermore, this study is the first to examine the leaching effects of biochar made from filtercake, which was shown here to attenuate DOC loss, likely through stabilization of high-molecular-weight, complex compounds already present in soil. However, filtercake biochar appeared to potentiate NO_3^- leaching losses. Because both percent C content and NO_3^- retention appear to increase markedly in high-temperature chars, it may be possible to improve both C sequestration and nutrient retention by pyrolyzing filtercake at a higher temperature. Given the practical benefits of

utilizing filtercake within its agro-industrial context, further investigation into biochar produced from this material and its effects on soil quality and sugarcane productivity are merited.

Chapter 4: Differential effects on carbon turnover due to residue bioavailability: implications for crop residue management in sugarcane

4.1 Introduction

Concern over climate change has greatly increased academic and non-academic interest in the role of soils in global carbon (C) sequestration. Given that soils contain twice the quantity of carbon as the atmosphere, which exists in the form of stabilized soil organic carbon (SOC) (Janzen, 2004), the role of the world's soils in sequestering C is enormous and has already been negatively impacted through agricultural activities (Lal, 2004). This has led to urgent calls to restore or augment soil C storage through a variety of mechanisms, including conservation agriculture (Machado and Silva, 2001), returning crop residues to cultivated fields (Metzger et al., 2002), and the use of recalcitrant C amendments such as biochar (Abiven et al., 2014).

However, the mechanisms through which C is incorporated into the soil, stabilized, and finally transformed into less bioavailable SOC are complex and the capacity of a given soil to store C is dependent on many site-specific factors such as texture, clay mineralogy and management (Stewart et al., 2007). The primary mechanisms that lead to the stabilization of soil C are thought to be chemical stabilization, physical protection, and biochemical stabilization, which have been used to loosely define five pools of soil C with varying residence times in the soil (Six and Conant, 2002). However, these pools are an acknowledged simplification of a complex system in order to allow modeling; the base assumption is that, once humified, SOC is less bioavailable and will change only slowly over time. This view has been challenged in recent years with the

development of research into *priming effects*, which refer to the increased turnover of soil C in response to fresh (non-humified, labile) C inputs (Kuzyakov, 2010).

Priming effects have a number of important implications for how we view soil C sequestration. First, this more dynamic perspective explicitly recognizes the actions of soil microbes and their enzymes as primary drivers of change in SOM stocks (Gleixner, 2013). In essence, fresh inputs, such as leaf litter, root exudates, or crop residues, alter the availability of substrate and trigger a succession of soil organisms (and increased CO_2 production) led initially by soil bacteria feeding off the new input, and later by soil fungi, feeding off the bacterial necromass and remaining soil C (Kuzyakov, 2010). Although in many cases priming effects do not affect total soil C stocks (Keith et al., 2011; Luo et al., 2011), they may affect the type of C stored, as increased fresh inputs and activation of the microbial community may lead to the oxidation of very old, humified C (Fontaine et al., 2007).

Because of this interaction between labile and recalcitrant C, the potential priming effects of biochar have also come under intense scrutiny. This is because although biochar is often posed as a means to increase the storage of stable, non-bioavailable C, numerous previous studies have observed that biochar increases soil microbial biomass (Chan et al. 2008; Steiner et al. 2008; Kolb et al. 2009), soil respiration or CO_2 efflux (Cross and Sohi, 2011; Novak et al., 2010; Zavalloni et al., 2011), and indeed induces a shift in the soil microbial community toward fungi (Anderson et al., 2011; Steinbeiss et al., 2009). This increase in soil microbial biomass and activity has been attributed to the presence of more labile bio-oils and tars on the surface of the char (providing an energy source), increased microhabitat within the pore space, or the effects of

biochar on increasing aeration (Lehmann et al., 2011). Furthermore, the magnitude of these priming effects are dependent on feedstock and pyrolysis conditions, and can last up to several years (Singh and Cowie, 2014). In addition, the stability of biochar itself may be affected by the presence of labile components in the soil, as previous studies have shown that increasing additions of fresh plant matter or glucose speed the mineralization of biochar itself (Hamer, 2004; Keith et al., 2011). Thus, despite the overall assumption that biochar amendment will mitigate soil C loss, there is potential for increased C turnover until the soil C fractions come to a new equilibrium.

Sugarcane bioethanol is interesting from this perspective because it involves intensive and repeated applications of crop residues to soils. As mentioned previously in this thesis, bioethanol production results in a number of residues, including bagasse, cane trash, vinasse, and filtercake. Of these, bagasse is burned for cogeneration of heat and electricity, and cane trash (*i.e.*, flags and leaves) may be burnt during harvest or allowed to decompose on the field, contributing to SOC stores (Cerri et al., 2011). However, vinasse and filtercake produced during processing are applied to the soil, and they may be applied at different loading rates in various combinations on different areas of the plantation, or not at all.[6] Furthermore, vinasse and filtercake are chemically distinct. Previous detailed characterization studies showed that sugarcane vinasse C is 80–90% DOC in the form of mono-, oligo-, and polysaccharides, as well as organic acids; the remaining 10–20% is particulate organic C made up of cellulose and hemicellulose, with small amounts of lipids and amino acids (Benke et al., 1998; Doelsch et al., 2009). Filtercake contains a larger

[6] The large size of sugarcane plantations and the fuel cost associated with hauling effluent and wet solid waste mean that vinasse and filtercake are most often heavily applied closest to the plant, whereas the furthest reaches of the plantation may receive nothing (George et al., 2010).

proportion of less labile carbon in the form of cellulose, hemicellulose, and lignin (**Section 2.3.1** and George et al., 2010). In contrast to vinasse and filtercake, filtercake biochar is primarily composed of aromatic carbon with residual carbohydrates (**Section 2.3.1**). Together, these residues represent a continuum of labile–recalcitrant material, and applying them alone or in combination may therefore differentially affect the pool of bioavailable carbon.

One way to trace the fate and effect of organic residues, without the expense of artificially ^{13}C- or ^{14}C-labelled substrates, is through the "natural abundance" or the $\delta^{13}C$ isotopic method. This method takes advantage of the differential accumulation of the ^{13}C *vs.* ^{12}C isotope in plants relying upon the C3 *vs.* C4 photosynthetic pathways, which leads to a measureable depletion or enrichment, respectively, of ^{13}C in the underlying soil and in plant tissue. This can be used to demonstrate how plant inputs affect the isotopic composition of SOC over the short or long term (Balesdent, 1988; Poirier et al., 2013). For example, the C4 plant sugarcane is enriched in ^{13}C (values at approximately –12‰), whereas the C3 vegetation of closed canopy Cerrado forest shows $\delta^{13}C$ values of approximately –28‰ (Miranda et al., 1997; Pessenda et al., 1998); these differences are likewise reflected in underlying soils. Analyzing the shift in soil $\delta^{13}C$ enrichment thus makes it possible to determine whether C lost from the soil is due to the mineralization of biochar, filtercake, or vinasse (resulting in ^{13}C depletion), or whether these substances have caused native SOC to be mobilized instead (enrichment in ^{13}C).

The objective of this chapter was to examine whether such changes in carbon quality, quantity and bioavailability due to crop residue management could potentially affect carbon turnover in sugarcane cultivation. This was examined through a combination of a CO_2 efflux assay, to assay

the relative bioavailability of these residues, alone or in combination, in a forest soil (compared to the cultivated soil discussed in **Chapter 2**), followed by total carbon and carbon turnover ($\delta^{13}C$) assays to assess the utilization of the various residue carbon sources, alone or in combination.

4.2 Materials and methods

4.2.1 Preparation of soil, sugarcane residues, and biochar

Mineral soil from a forested riparian area was collected from Usina Pantanal, a sugarcane plantation in Jaciara, MT, Brazil (15°55'28.11"S, 55°13'38.94"W, elevation 690 m). A forest soil was used for all analyses in this chapter because of its naturally low ^{13}C isotope levels, which allowed carbon from this source to be more easily distinguished from organic material deriving from the ^{13}C-enriched sugarcane residues.[7] The top 0–10 cm of the bulk soil was collected from a single soil pit under closed canopy gallery forest at the beginning of the wet season. To minimize any change in the soil microbial community, soil was transported on ice to the laboratory, where it was stored in a refrigerator until use. The soil was classified as an Arenosol in the FAO soil taxonomy with a very sandy texture (90% sand, 1% silt, 9% clay).

Sugarcane distillation by-products were also obtained from Usina Pantanal. Filtercake was dried at 45°C and sieved to < 2 mm for use in soil treatments. Filtercake biochar, the production of which was described in detail in **Chapter 2**, was also sieved to < 2 mm. Vinasse was collected from canals within the sugarcane production area close to the point of field application and kept

[7] This is in contrast to the cultivated soils used in **Chapters 2** and **3**, which were expected to be relatively more enriched in ^{13}C due to the fact that they have been under cultivation with a C4 plant (sugarcane) for many years.

frozen at −20°C prior to application to the CO_2 efflux or $\delta^{13}C$ incubations. The carbon and nitrogen contents of the forest soil, lyophilized vinasse, biochar, and filtercake were determined on a CHN-1110 elemental analyzer (Carlo Erba Instruments, Italy). Next, pH and oxidation-reduction potential (ORP) for the solid materials were determined by mixing 3 g of material in 27 g of water (Lee et al., 2013), followed by 30 min of intermittent shaking, 30 min of settling, and measurement with a handheld meter (HI 98121, Hanna Instruments, USA). Surface area data are as described in **Chapter 2.** Biochar, filtercake, and vinasse characteristics are reported in **Table 4.1**.

Residue	***Forest Soil***	***Biochar***	***Filtercake***	***Vinasse***
pH	4.6 ± 0.1	9.9 ± 0.1	5.9 ± 0.0	5.2 ± 0.1
ORP (mV)	226. ± 12	40 ± 3	168 ± 5	132 ± 3
Total C (g kg^{-1})	18.6 ± 0.1	383 ± 3	375 ± 9	1.29 ± 0.04 g L^{-1}
Total N (g kg^{-1})	0.9 ± 0.0	15.4 ± 0.1	14.4 ± 0.2	0.08 ± 0.00 g L^{-1}
C:N	19	28	26	16
Surface area (m^2 g^{-1})	N.D.	26	0.38	N.D.

Table 4.1. Physicochemical characterization of biochar, filtercake, vinasse, and a forest soil.

*** Biochar pH and oxidation-reduction potential (ORP were taken in water. N.D., not determined).**

4.2.2 CO_2 efflux assay

In contrast to the soil treatments described in **Chapter 2**, soil treatments used throughout this chapter were established as follows: soil alone (S); soil + 0.5% filtercake (by weight, SF); soil + 5% biochar (by weight, SB). The quantities of filtercake and vinasse applied were equivalent to field application rates of roughly 5 Mg ha^{-1} and 100 m^3 ha^{-1}, respectively. The absolute and relative contributions of soil and each of the residues to total initial C content are shown in **Table 4.2**.

	Treatment					
	S	***SV***	***SF***	***SVF***	***SVB***	***SB***
Treatments for CO_2 Efflux Assays						
Soil	17.3 (100%)	17.3 (99.5%)	17.6 (91.7%)	17.6 (91.2%)	16.8 (45.2%)	16.8 (45.3%)
Filtercake	0	0	1.6 (8.3%)	1.6 (8.3%)	0	0
Biochar	0	0	0	0	20.3 (54.6%)	20.3 (54.7%)
Vinasse	0	0.01 (0.5%)	0	0.1 (0.5%)	0.1 (0.2%)	0
Total Initial C	**17.3**	**17.4**	**19.1**	**19.3**	**37.3**	**37.2**
Treatments for $\delta^{13}C$ Assay						
Soil	4.0 (100%)	4.0 (86.0%)	4.0 (92.7%)	4.0 (80.6%)	4.0 (49.7%)	4.0 (54.1%)
Filtercake	0	0	0.3 (7.3%)	0.3 (6.3%)	0	0
Biochar	0	0	0	0	3.4 (42.2%)	3.4 (45.9%)
Vinasse	0	0.6 (14.0%)	0	0.6 (13.1%)	0.6 (8.1%)	0
Total Initial C	**4.0**	**4.6**	**4.3**	**4.9**	**8.0**	**7.4**

Table 4.2. Initial treatment C contents and absolute (g) and relative (%) contributions of each component.

To prepare these treatments, fresh (refrigerated), field-moist soil was sieved to 4 mm, roots were removed by hand, and then the soil was gently mixed to ensure a uniform composition. Filtercake or biochar was added as required during mixing, and soil for all treatments was handled to an equal degree. Equal weights (1200 g) of these treatments were packed into PVC incubation columns (10 × 15 cm, D × L; $n = 3$ each) and left open to the atmosphere in a temperature-monitored environment. After a 24-h equilibration period, CO_2 effluxes were measured daily using a LI-COR 6400 XT apparatus (LI-COR, Lincoln, NE) as described in **Chapter 2**. Measurements were collected over three weeks under three conditions: week 1, field-moist (no added water); week 2, 30 mL water or vinasse (low dose); week 3, 60 mL water or vinasse (high dose). The maximum amount of liquid that could be added (50 mL) was limited in order not to surpass 60% water-holding capacity. Vinasse and water were applied by slowly and uniformly dripping liquid over the soil surface using a pipette and then mixing with a clean

spatula. Incubations were carried out at ambient temperature, ranging from 25–29°C during the night and 29–35°C during the day.

4.2.3 $\delta^{13}C$ incubations and analysis

$\delta^{13}C$ analyses were used to determine the effect of biochar on the carbon turnover or potential priming of SOC, in comparison to treatment with organic residues alone. Accordingly, a field-moist C3 soil from a forested area and sieved to 4 mm in the lab in preparation for soil incubation. The same soil treatments as described in **Section 4.2.1** were used in the $\delta^{13}C$ study, although vinasse C, where applied, made a greater overall contribution to treatment C content. The absolute and relative contributions of soil and each of the residues to total initial C content are shown in **Table 4.2**. Briefly, soil (S) with or without 0.5% filtercake (SF) or 5% biochar (SB) were weighed into plastic incubation pots (240 g each) and closed with perforated lids. Three analytical replicates plus an additional replicate for microbial analyses (enzyme and microbial biomass assays shown in **Appendix F**) were prepared for each treatment. At the beginning of the experiment, all pots were adjusted to 50% water-holding capacity with either 50 mL water (S, SF, and SB) or 50 mL vinasse (SV, SVF, and SVB). Thereafter, water content was adjusted gravimetrically every two weeks as the containers were incubated in an enclosed, darkened closet at 30 ± 5°C for 90 days.

At the end of the incubation period, day 0 and day 90 samples were dried at 45°C for 2 days and the dried soil samples sent to the Centro de Energia Nuclear na Agricultura (CENA) at the University of São Paulo for isotopic analysis. Analyses were carried out in triplicate on a Thermo

Quest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT; analytical precision, ±0.1‰) coupled with a CHN-1110 elemental analyzer (Carlo Erba Instruments, Italy; analytical precision, ±0.02%) for % C determination. The fraction of the ^{13}C against the ^{12}C isotope was normalized against an internationally recognized standard (Pee Dee Belemnite) as follows:

$$\delta^{13}C\ ‰ = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 100$$

Equation 4.1.

where, R_{sample} and $R_{standard}$ refer to the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. Next, the fraction of SOC deriving from sugarcane residue was calculated as follows:

$$F_{residue} = \frac{(\delta_{sample} - \delta_{control})}{(\delta_{residue} - \delta_{control})}$$

Equation 4.2

where δ_{sample}, $\delta_{control}$, and $\delta_{residue}$ refer to the $\delta^{13}C$ values of the amended sample, the unamended day 0 or day 90 control, and the pure residue, respectively. For combination treatments, a weighted $\delta^{13}C$ value was used based on the C contribution of each of the residues.

4.2.4 Statistical analyses

All data are presented as the mean ± standard error (SE). In the CO_2 efflux assay, total g CO_2-C emitted over the course of the experiment for each treatment was compared via one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey test. In the $\delta^{13}C$ assay, differences between

treatments at 0 *vs.* 90 days were analyzed via unpaired, two-tailed *t*-tests. A *p*-value of 0.05 was considered statistically significant. All statistical analyses were carried out in R v.3.0.1 (R Core Team, 2013) using Rcmdr (Fox, 2005).

4.3 Results

4.3.1 CO_2 efflux assay

A CO_2 efflux assay was used to examine the effect of amendments on forest soil respiration, alone or in combination, which was useful to determine the relative bioavailability of each residue and whether positive or negative priming was observed relative to the amount of C added. As shown in **Fig. 4.1** and **Table 4.3**, filtercake addition resulted in a 27-fold increase in total soil CO_2 efflux relative to the soil control over the course of the experiment. In contrast, biochar addition resulted in only a 3.5-fold increase relative to the soil-only control, despite the fact that it was applied at a 10-fold greater dose. Vinasse addition (SV, SVF, and SVB) had no effect relative to samples treated with water (S, SF, and SB) regarding either total g CO_2 lost or % initial C lost.

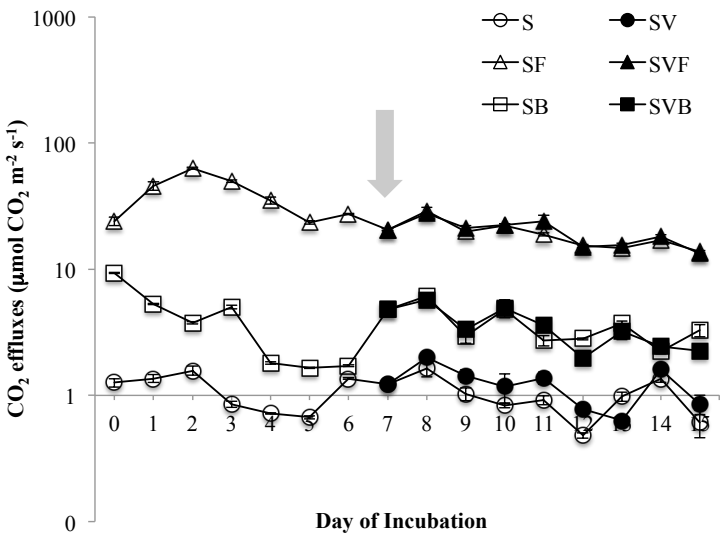

Figure 4.1 Short-term CO_2 effluxes from a residue-amended forest soil.

Data points represent the mean treatment value ± SE (*n* = 3). Note that untransformed data have been plotted on a logarithmic scale. Water (open symbols) or vinasse (solid symbols) was added on day 7. S, soil; SV, soil with vinasse; SF, soil with 5% filtercake (d.w.); SVF, soil with 5% filtercake and vinasse; SVB, soil with 5% biochar and vinasse; SB, soil with 5% biochar.

Treatments	*Total CO_2 emitted (g CO_2)*	*Initial C content (g C)*	*Initial C lost (%)*
S	0.46 ± 0.02, *a*	17.3	0.73 ± 0.02, *a*
SV	0.54 ± 0.02, *a*	17.5	0.85 ± 0.03, *a*
SVB	1.64 ± 0.13, *b*	37.3	1.20 ± 0.04, *b*
SB	1.65 ± 0.04, *b*	37.2	1.21 ± 0.03, *b*
SF	12.5 ± 0.2	19.2	17.7 ± 0.4
SVF	12.8 ± 0.1	19.3	18.1 ± 0.2

Table 4.3. CO_2 emitted and percent initial carbon lost from forest soil.

To examine differences among S, SV, SVB, and SB, total CO_2 emitted and % C lost were compared via one-way ANOVA. Data represent the mean ± SE. Total C content refers to the sum of initial carbon present in the soil plus C added through amendments. Lowercase letters in common indicate non-significant differences between treatments as determined using Tukey HSD test. Because filtercake addition greatly skewed the data set, SF and SVF treatments were analyzed independently using a two-tailed unpaired *t*-test and found not to differ significantly. Note that data for g CO_2 emitted did not satisfy the Shapiro–Wilk normality test at 0.05.

When contrasting CO_2 effluxes against the cultivated soil examined in **Chapter 2**, it is interesting that percent C lost followed roughly the same pattern in forest soil that it did in cultivated soil (**Fig. 4.2**). Overall, the forest soil lost a greater proportion of initial soil C, which likely reflects the fact that C in this sandier soil is more vulnerable to degradation than in the cultivated soil with a higher clay content, which confers greater physical and chemical stabilization (Six and Conant, 2002).

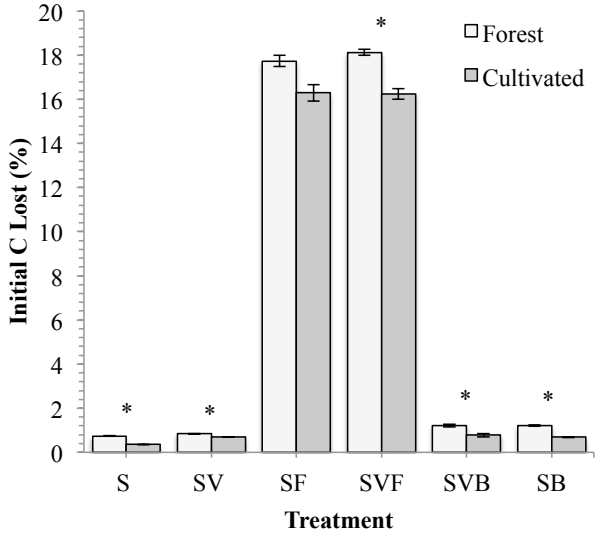

Figure 4.2. Comparison of % initial C lost in the residue-amended forest *vs.* cultivated soil.

Data represent the mean ± SE (*n* = 3). For each treatment group, means between the forest *vs.* cultivated soil were compared using an unpaired *t*-test assuming equal variance.

4.3.2 Carbon turnover in residue-amended forest soil

To examine the effect residues on C turnover, soils were amended with various combinations of vinasse, filtercake, and biochar and then incubated in the dark for 90 days. Before and after this

incubation period, soils were analyzed to determine total soil C contents and to determine the fraction of sugarcane-derived C (from vinasse, filtercake, or biochar) within the soil. Several interesting trends were observed that suggest shifts in the source of soil C utilized under different residue treatments. Before incubation, soil C analyses revealed that amendment with vinasse (50 mL) and/or raw filtercake (0.5% d.w.) had no observable effect on soil C content relative to the unamended control (**Fig. 4.3** and **Table 4.4**), owing to the relatively low doses of these amendments and potential heterogeneity introduced when incorporating amendments. Nevertheless, the addition of these seemingly insignificant amounts of C appeared to elicit increases in soil C contents after 90 days in the SF and SVF treatments, indicating an unexpected increase in C fixation in soils incubated in the dark (referred to here as 'dark fixation') (Shimmel, 1987). The addition of biochar, in contrast, increased initial C content relative to the untreated soil, but had no discernible effect on C content on 90 days *vs.* 0 days, with or without vinasse co-application.

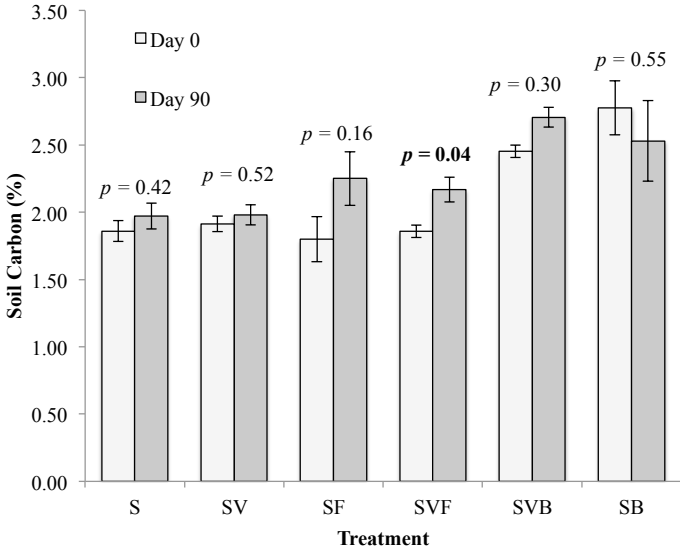

Figure 4.3. Carbon contents in residue-amended forest soil after 90 days of incubation.

Data represent the mean ± SE. For each treatment group, means between Day 0 *vs.* Day 90 were compared using an unpaired *t*-test assuming equal variance (*n* = 3).

Treatment	*Total Carbon (%)*	*$\delta^{13}C$ Value (‰)*	*Residue Fraction*
Day 0			
S	1.86 ± 0.08, *a*	–27.25 ± 0.06, *a*	- -
SV	1.91 ± 0.06, *a*	–27.92 ± 0.05, *a*	–0.01 ± 0.00, *a*
SF	1.80 ± 0.17, *a*	–27.07 ± 0.25, *a*	0.05 ± 0.02, *a*
SVF	1.86 ± 0.05, *a*	–26.74 ± 0.53, *a*	0.07 ± 0.04, *a*
SVB	2.45 ± 0.20, *ab*	–22.77 ± 0.14, *b*	0.34 ± 0.01, *b*
SB	2.78 ± 0.24, *b*	–22.93 ± 0.08, *b*	0.33 ± 0.01, *b*
Day 90			
S	1.97 ± 0.10, *A*	–27.85 ± 0.11, *A*	- -
SV	1.98 ± 0.08, *A*	–27.50 ± 0.16, *A*	0.02 ± 0.01, *A**
SF	2.25 ± 0.20, *A*	–27.62 ± 0.03, *A*	0.02 ± 0.00, *A*
SVF	2.17 ± 0.09, *A*	–27.38 ± 0.05, *A*	0.03 ± 0.00, *A*
SVB	2.71 ± 0.07, *A*	–22.45 ± 0.30, *B*	0.37 ± 0.02, *B*
SB	2.53 ± 0.30, *A*	–22.52 ± 0.06, *B**	0.37 ± 0.00, *B**

Table 4.4. Complete C utilization data for forest soil at 0 and 90 days of incubation.

Data represent the mean ± SE (*n* = 3). In-common lowercase or uppercase letters indicate non-significant differences among treatments for separate analyses within the Day 0 and Day 90 data sets, respectively, as determined via oneway analysis of variance (ANOVA) followed by a *post hoc* Tukey test. Regarding the effect of time, asterisks beside values in the Day 90 treatment indicate significant differences with respect to the same treatment in the Day 0 data set.

In the second part of the analysis, $\delta^{13}C$ data were used to determine the fraction of a given sugarcane residue (vinasse, filtercake, biochar, or a combination thereof) within the sample before and after incubation for 90 days (**Fig. 4.4** and **Table 4.3**). $\delta^{13}C$ values for residues were –12.14‰, –13.32‰, and –13.26‰ for vinasse, filtercake, and biochar, respectively. The analysis revealed that the residue fraction deriving from vinasse appeared to increase after 90 days (SV, $p = 0.07$, **Fig. 4.4, Table 4.3**). After 90 days, the residue fraction deriving from filtercake appeared to decrease (SF and SVF), perhaps indicating the preferential utilization of filtercake by soil microbes. In contrast, in the biochar treatments (SVB and SB), the residue fraction appeared to increase over time, indicating that native soil C was utilized preferentially over the biochar C.

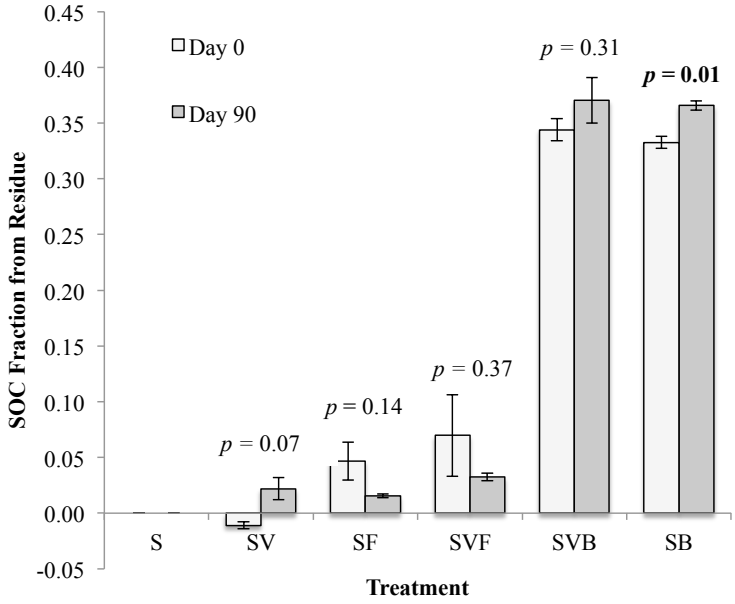

Figure 4.4. Changes in the fraction of total SOC derived from sugarcane residue over 90 days.
Soils treated with vinasse, filtercake, biochar, or combinations of these were subjected to $\delta^{13}C$ analyses before incubation (day 0) and after 90 days of incubation in the dark, which was used to assess the initial and remaining fraction of that carbon. Please note the data presented here (mean ± SE, $n = 3$) are dimensionless values based on Equation 4.2 in Section 4.2.3.

4.4 Discussion

4.4.1 Effects of residue amendments on soil CO_2 effluxes

Our study revealed that biochar amendment greatly reduced CO_2 efflux from a forest soil compared to raw filtercake amendment. Biochar addition still increased total C loss from the soil to from 0.73 ± 0.02% in the control to 1.21 ± 0.03% in the biochar-amended soil. This may suggest that biochar, through changes to soil pH, water balance, substrate availability, or increased aeration, has provoked an increase in microbial activity. Numerous previous studies have reported similar increases in CO_2 effluxes, although it is not clear what proportion of this efflux is biologically *vs*. abiotically mediated (Cross and Sohi, 2011; Jones et al., 2011; Novak et al., 2010; Zimmerman, 2010). Although some studies have shown an increase in microbial biomass associated with biochar, other studies have shown that abiotic mechanisms can account for up to 50–70% of total CO_2 released; these mechanisms may include off-gassing, to a limited extent, decarboxylation reactions catalyzed by the biochar surface, or acid-base reactions occurring between the alkaline biochar and the acidic soil solution (Jones et al., 2011; Zimmerman, 2010). Given that the soil in question was quite acidic (pH 4.0 ± 0.1) and our biochar quite alkaline (pH 9.9 ± 0.1), there is a strong possibility of the latter occurring. Therefore, in the absence of a reliable microbial biomass C determination (for reasons described in **Appendix F**), it is not possible to determine whether the elevated CO_2 efflux in our study was the result of expansion of the microbial community or its utilization of SOC.

4.4.2 Carbon utilization results

Trends in the $\delta^{13}C$-derived data followed from the chemical characterization of these materials presented in **Chapter 2**, although their interpretation is complicated by the occurrence of dark C

fixation as described above. Shifts in the sample $\delta^{13}C$ value toward more negative values (depletion) should indicate utilization of sugarcane C, whereas positive shifts (enrichment) should indicate the removal of native SOC, equating to decreases and increases, respectively, in the residue fractions shown in **Fig. 4.4**. However, the unexpected fixation of atmospheric C (with a $\delta^{13}C$ value of approx. –8‰) could induce a shift toward more positive values, appearing as native SOC utilization. Thus, treatments showing trends toward dark C fixation (SF and SVF) in the total C analyses must be interpreted carefully.

Overall, the $\delta^{13}C$ data used to calculate residue fractions (**Fig. 4.4),** suggest the occurrence of a priming effect in both the SF and SB treatments; this is defined as increased C turnover (*i.e.*, respiration) in response to C input (Kuzyakov, 2010), which may or may not also lead to an overall change in C stocks. Filtercake C in the SF treatment was preferentially utilized over native soil C. However, because the SF treatment appeared to show dark C fixation, thus artificially elevating the $\delta^{13}C$ value, this effect is likely more pronounced than observed in **Fig. 4.4** (*i.e.*, even greater filtercake utilization). The $\delta^{13}C$ analyses also suggest that native C was preferentially utilized over biochar C (SB treatment, **Fig. 4.4**), indicating that the increased CO_2 fluxes observed here are not due primarily to the mineralization of volatile materials on biochar, as suggested by some authors (Cross and Sohi, 2011), but rather reflect an increase in existing soil C turnover. This is likely because this filtercake biochar was produced at well above 400°C, at which point biochars show decreasing amounts of remnant volatile material post-pyrolysis (Novak et al., 2009b). This is not to say that biochar C is inert in the soil, but does indicate that it is more stable than the least stable elements in the soil. Importantly, however, vinasse in combination with either filtercake or biochar did not show this trend (SVF and SVB treatments,

Fig. 4.4). Previous work has shown that the co-application of biochar with a highly bioavailable substance can substantially increase the mineralization of supposedly 'stable' C in biochar (Hamer, 2004) or lignin (Hamer and Marschner, 2002). This does not appear to be the case with either filtercake (SVF) or filtercake biochar (SVB) applied with vinasse.

Finally, the trend toward increased soil C storage in response to vinasse application in **Fig. 4.4** suggests that the vinasse C fraction increased in the soil after 90 days, even though chemically this substrate should be extremely labile and did not make a significant contribution to total soil C in **Fig. 4.3.** It is instead more likely that this reflects an increase in the $\delta^{13}C$ ratio due to the dark fixation of atmospheric C with a $\delta^{13}C$ value of –8‰; small changes like this one are more likely to be detected via the highly sensitive $\delta^{13}C$ analysis, even if they do not appear in the less-sensitive total C analysis. Although the difference between day 0 and day 90 for the SV treatment did not reach statistical significance ($p = 0.07$), the data may indicate that the presence of dissolved substances within vinasse stimulates the activity of soil microbes involved in non-photoautotrophic C fixation, for which a variety of mechanisms have been proposed (Miltner et al., 2004). Alternatively, if dark C fixation is not the source of this anomalous trend, it may indicate that vinasse constituents stimulate the oxidation of native C, which again may be evidence of a priming effect.

Total C analyses revealed that, overall, filtercake and vinasse contributed little to the starting C content, whereas biochar contributed greatly, owing to the doses applied in this study (see further discussion in **Section 4.4.3**). We also observed a trend toward increased C content in the SF and SVF treatments after 90 days, even though these incubations did not include plant life and were

incubated in the dark to exclude even the small potential contribution of soil photoautotrophs. The notion of dark C fixation may at first seem surprising, given that bare or cultivated soils lacking plants and their inputs are generally considered to be sources of CO_2 to the atmosphere (Schlesinger and Andrews, 2000). However, CO_2 effluxes are the net effect of multiple simultaneous processes, including biotic respiration by soil microbes, abiotic oxidation of C compounds, as well as a small but detectable amount of C fixation carried out by both plants and soil microorganisms. Previous work has demonstrated increases in soil C content due to non-photosynthetic C fixation in samples incubated in the dark under an atmosphere enriched in $^{13}CO_2$ (Miltner et al., 2005). Furthermore, the incorporation of ^{13}C in amino sugars, amino acids, and specific fatty acids revealed that this increase is C content is mediated via the soil microbial biomass, principally gram-negative bacteria and actinomycetes (Miltner et al., 2005). Thus, in this study and previous work, non-photosynthetic C fixation carried out by soil microbes may elicit small but detectable C increases even in dark-incubated soils.

4.4.3 Resolving doubts regarding the effect of residues on soil carbon priming

The C utilization study described here consisted of soil C and $\delta^{13}C$ analyses that were intended to examine the contribution of the residues (vinasse, filtercake, and biochar) to total C content and to assess the differential utilization and/or co-mineralization of these residues, presumably due to their bioavailability. Several approaches could be taken to improve the quality of data from this assay, and to better demonstrate the individual and combined effects of sugarcane residues on C turnover. One approach would be to apply equal amounts of C from each residue rather than the moderate doses used here. Although the loadings used here were based on the amount of vinasse or filtercake that could be produced from the same hectare of sugarcane, field applications are

often much higher over smaller areas. For example, vinasse applications often greatly exceed the legal limit of 300 m^3 ha^{-1} (Cruz et al., 2011; Passarin et al., 2007), and filtercake may be applied at up to 80–100 Mg ha^{-1} over small areas (Prado et al., 2013). With equal C loading, it may have been possible to better appreciate changes in total and residue C contents, and to determine whether vinasse in combination with filtercake or biochar does indeed potentiate native soil C oxidation. Furthermore, such an approach would avoid potential issues with C saturation effects, which refers to the fact that initial soil C content can affect turnover and stabilization of added C (Kimetu et al., 2009; Poirier et al., 2013). An overall increase in C loading would have also rendered negligible the effects of dark C fixation and any related complications in interpreting $\delta^{13}C$ data. Following the experiment for a longer period may have allowed C changes to accumulate, although it should be noted that short incubation periods (50–90 days) are routinely used in other studies of this kind (Miltner et al., 2005; Poirier et al., 2013). Finally, in order to most effectively demonstrate a priming effect, measurement of the residue fraction in respired CO_2 is also recommended, along with microbial biomass C determination (Kuzyakov, 2010). This approach allows a full mass balance for C, including CO_2 released from residue, CO_2 released from SOM, and the amount of C stored as active biomass, as well as residue and SOM fraction remaining in the soil. Unfortunately, $^{13}CO_2$ determinations were not feasible given the research location; microbial biomass determination, furthermore, was complicated by the presence of highly adsorbent filtercake biochar (**Appendix F**).

4.4.4 Summary

The objective of this study was to examine the effect of filtercake biochar on soil respiration, and to probe for potential interactions among crop residues applied in combination compared to each

residue applied alone. Although few significant effects were found, due to the short incubation period, these data do demonstrate that each of the sugarcane-derived residues examined here show differential effects on soil C turnover, which may have implications for management. Filtercake biochar, in particular, was found to induce a small but significant increase in soil CO_2 efflux relative to the unamended soil, and the source of this lost C was mobilized soil organic matter. However, co-application with a labile residue (vinasse) did not appear to exacerbate co-mineralization, suggesting that this biochar is relatively stable in the soil.

Chapter 5: Conclusions

5.1 Summary of experimental results

The overall aim of this thesis was to re-examine filtercake as a potential feedstock for biochar, as outlined in the research questions listed in **Section 1.4**. In **Chapter 2**, the production and characterization of filtercake biochar is described. Although several previous studies have examined bagasse biochar, this was the first to characterize filtercake biochar, which is a much more practical approach to integrating biochar with bioethanol production given the high value of existing bagasse resources (discussed further in **Section 5.2.3**). As expected, filtercake biochar elicited improvements in soil pH, cation exchange capacity (CEC), and nutrient availability (P, K, Mg, Ca, Mg, Fe, Mn, and Zn) relative to an unamended soil. A significant effect was also observed on water retention at 5 and 10% d.w. biochar. These results are generally in line with previously published results (Basso et al., 2013; Biederman and Harpole, 2012; Jeffery et al., 2011), although the magnitude of effects is impossible to compare directly given the differences in soil types and application rates used. In addition, compared to raw filtercake, filtercake biochar also greatly decreased the amount of CO_2 lost to rapid mineralization upon first incorporating residues into the soil. However, the small but significant observed increase in CO_2 efflux relative to the untreated control suggested that biochar activates soil microbial respiration and carbon (C) turnover, suggesting against a toxic effect at this high dose, as has been observed with other specific biochars (Rajkovich et al., 2011; Solaiman et al., 2011). Thus, although this "first look" at biochar did not include plant assays, it provided an abundance of valuable information that strongly supports further investment to examine effects on a larger scale (germination, pot, and field studies) as described in **Section 5.3.1**.

In **Chapter 3**, it was shown that the inclusion of biochar significantly attenuated the loss of dissolved organic carbon (DOC) from a cultivated Ferralsol, with or without application of vinasse. Fluorescence and absorbance spectroscopy indicated that filtercake biochar preferentially retained larger and more complex, humified DOC species, whereas less complex species and amino acid-like compounds were not affected by the presence of biochar. Despite the diversity of optical indices used, this pattern was consistent across indices, lending strength to the conclusion that aged biochar in the soil retained complex DOC species. Generally, studies examining biochar and soil DOC tend to focus on biochar as a source of DOC and fail to recognize that the interaction between charred material and soluble C compounds observed in nature could mean that biochar might assist in DOC retention and perhaps stabilization (Kalbitz et al., 2005). Thus, these data fill an important gap in the literature regarding the effect of biochar on DOC retention, an area in which very little other work has been done (a notable exception: Kasozi et al., 2010). However, it would take further study over much longer periods with more sophisticated chemical characterization to determine whether or not this DOC is semi-permanently stabilized or mineralized. Regardless, the observed decrease in leaching limits at least one avenue for C loss from soils, decreasing the risk of negative impacts from vinasse application on nearby waterways.

However, biochar inclusion in leaching columns did *not* slow leaching of another important vinasse constituent, NO_3^-. This is important because the ability to halt the leaching of this critical nutrient from soils is widely used to justified pursuing biochar research, even though there is in fact very little evidence to support NO_3^- leaching in soil–biochar mixtures that have been allowed

to age (see **Section 3.4.1**). "Ageing" refers to weathering or oxidation of biochar that occurs in a moist environment, whether that be moist air or, for a much greater effect, mixed with moistened soil containing organic matter (Joseph et al., 2010). In fact, decreased bulk density and increased hydraulic conductivity in biochar-amended soils appears to have exacerbated NO_3^- leaching in this experiment. This contribution is significant because it demonstrates the importance of avoiding the use of mixtures of soil and fresh biochar in such characterization assays, as this approach ignores the dynamic *in natura* interaction between soil components and charred material that biochar research is in some ways attempting to replicate.

Finally, in **Chapter 4**, CO_2 efflux, total soil organic carbon (SOC), and $\delta^{13}C$ analyses were used to examine the turnover of biochar *vs.* native SOC in a forest soil. Trends in the data suggest that application of the various residues derived from sugarcane processing result in differential patterns in C turnover in an uncultivated or native soil. Where raw filtercake appeared to be mineralized preferentially over native SOC, biochar application appeared to provoke mineralization of native SOC. These results suggest that biochar is not inert in the soil, but rather that biochar could be stimulating C turnover in a way that may be beneficial (through release of plant nutrients) or negative (through priming the loss of stored C). The data also suggest that although vinasse C may be an essentially negligible contribution to total SOC, the effect of its other constituents may have a stronger effect on SOC turnover than expected, driving an apparent increase in content after 90 days of dark incubation compared to initial C content.

This project also encountered several obstacles and limitations. A key obstacle in completing the work outlined in **Research Question 4** is that filtercake biochar had very notable, unforeseen

impacts on two standard methodologies (colorimetric soil enzyme assays and chloroform fumigation–extraction) that were unresolvable within the time available. Potential solutions to this issue are presented in **Appendix F**. In addition, it would have been useful to extend the incubation periods for the CO_2 efflux and $\delta^{13}C$ incubations. In the former, this would have allowed additional modeling activities to determine the mean residence time of biochar, which is a useful parameter when comparing among chars in the literature. Regarding the $\delta^{13}C$ incubations, the lack of significant effects observed are likely due to insufficient time for SOC changes to accumulate, as discussed in **Section 4.4.3.**

5.2 Potential impacts in the sugarcane industry

Integrating biochar with the sugarcane industry has large potential benefits deriving from increases in sugarcane productivity, decreased agronomic inputs, increased energy generation, and the sale of C credits. Each of these will be addressed in the following sections.

5.2.1 Increases in productivity and soil remediation

Very little peer-reviewed or project work has been published on the effects of biochar amendment on sugarcane. In the single study published to date, sugarcane plots grown in soil amended with bagasse biochar (at a rate of 3% d.w. of the top 0.3 m) showed increased stalk weight, increased stalk yield (kg ha^{-1}), and an almost two-fold increase in the estimated sugar yield compared to untreated soil (Chen et al., 2010). These effects were associated with an increase in soil WHC and decreased NO_3^- leaching, although these effects were likely the result of increased biomass productivity rather than retention on or within biochar. As noted in

Chapter 3, the effects of biochar on NO_3^- retention are highly variable and unlikely to persist over time as biochar ages (oxidizes) in the soil.

However, despite the lack of previous studies, one would expect biochar amendment to have relatively large positive effects on sugarcane growth, for two reasons. The first is the observed increases in productivity in related C4 grasses and other food crops, including maize, sorghum, and millet. Numerous studies have looked at the effect of biochar on maize, and have collectively shown a small (~5%) but relatively consistent increase in plant productivity over 54 data points (as reviewed in Jeffery et al., 2011). In a greenhouse study looking at the effects of 32 biochars (eight feedstocks pyrolyzed at four temperatures) applied at four different application rates (0.2, 0.5, 2.0, 7.0%), the majority of treatments (100 of 128) showed positive effects on maize biomass, with increases up to 50%[8] of the control (Rajkovich et al., 2011). Of the 28 experimental treatments showing negative effects on biomass, 22 involved biochars from specific chars (dairy manure, food waste, and paper mill waste) that were applied at the highest doses of 2 and 7%. In the field, a single application of ~3% wood biochar led to large maize yield increases (28, 30, and 140%) over three consecutive years (Major et al., 2010). These increases were attributed to a large increase in Ca and Mg availability, which was also observed with filtercake biochar amendment (**Chapter 2**, **Table 2.1**). Interestingly, biochar effects on productivity seem to increase with passing years (Jones et al., 2012; Major et al., 2010b). In millet production, biochar from sewage sludge, de-inking sludge, *Miscanthus,* or wood applied at 3% d.w. elicited 1100, 200, –60, and –20% changes in dry shoot mass (Paz-Ferreiro et al., 2013). In contrast, sorghum biochar had no effect on sorghum biomass, perhaps due to the very small

[8] Percent change values estimated from graphical data presented in Figure 1 of the original paper.

applications rates used (0.07 and 0.14%)[5] (Schnell et al., 2012). Steiner et al. (2007) observed a two-fold increase in grain yield using a slightly higher biochar application (0.5%).[9] These data demonstrate the generally positive effects of biochar amendment on productivity in C4 plants similar to sugarcane, as long as an appropriate char type is applied at an appropriate dose (0.5–3%).

Figure 5.1. An Arenosol used for sugarcane cultivation at Usina Pantanal, MT, Brazil.

The second reason that biochar would be expected to increase sugarcane productivity is that biochars typically have their greatest benefits in the poorest soils (Biederman and Harpole, 2012; Jeffery et al., 2011). Sugarcane is often grown on infertile soils, and particularly so in the state of Mato Grosso where this work was carried out (**Fig. 5.1**). In fact, only 1.3% of the recent sugarcane expansion in the state of Mato Grosso has occurred on newly converted, more fertile land; the majority of new sugarcane (68%) has occurred on abandoned soybean fields (as

[9] Assuming a soil bulk density of 1.4 g cm^{-3} and an incorporation depth of 15 cm.

described in Lisboa et al., 2011). However, Cerrado soils are less fertile than the traditional sugarcane-producing regions of Brazil; Mato Grosso is dominated by nutrient-poor Ferralsols and Arenosols and has a mean annual sugarcane production of 68 Mg ha^{-1}, whereas the prime sugarcane-producing region of São Paulo is also dominated by Ferralsols, but has a greater proportion of clayey Acrisols and shows yields of 80 Mg ha^{-1} (FAO, 2004; SIDRA, 2014). Water limitations also play an important role. Although both regions receive roughly the same amount of precipitation (1515 ± 161 mm in Mato Grosso *vs.* 1463 ± 115 mm in São Paulo in 2013; (BDMEP, 2013b)), Mato Grosso experiences a marked dry spell between May and October, occurring during the early part of sugarcane's growth phase. In some regions, infrastructure such as vinasse canals and pumping stations can help to relieve water stress through fertigation; however, fertigation is often limited to areas close to the plant (Prado et al., 2013). Thus, despite sugarcane's impressive natural hardiness,[10] chemical inputs (*e.g.*, lime and fertilizers), vinasse fertigation, and (less frequently) irrigation are often used to maximize productivity, which carry high economic and environmental costs (Martinelli and Filoso, 2008). Biochar use in sugarcane cultivation may help to reduce these regional soil and water limitations on sugarcane productivity, although the degree to which that is possible must be the subject of further investigations (**Section 5.3.2**).

[10] This hardiness stems from both its C4 metabolism, which confers enhanced photosynthetic water use and nitrogen use efficiency (Chapin et al., 2011), as well as its capacity for endophytic nitrogen fixation (Boddey et al., 1991).

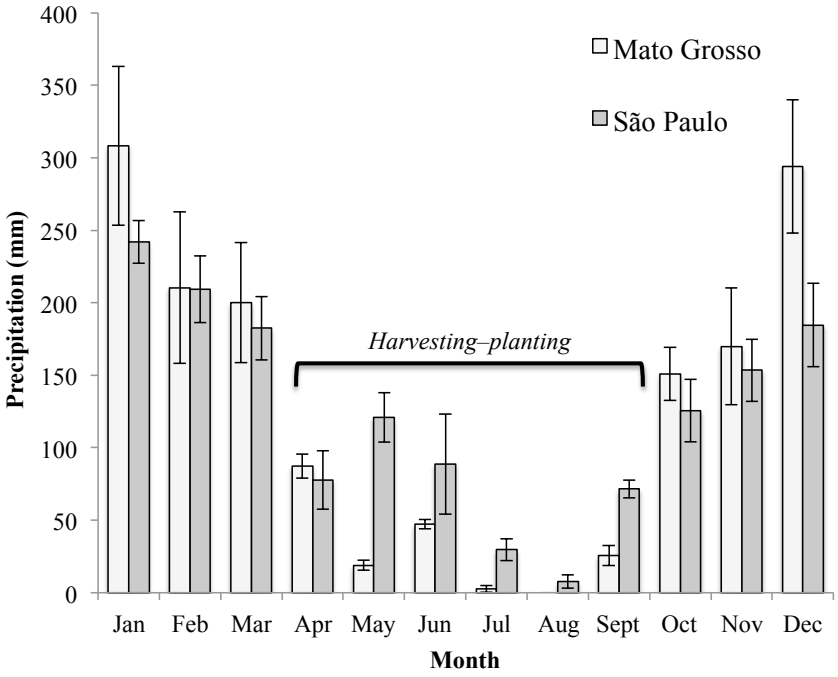

Figure 5.2. Total monthly precipitation for Mato Grosso and São Paulo in 2013.

Total monthly precipitation (mm) for each state was obtained by averaging monthly data from all available meteorological stations within the primary sugarcane-growing region of each state. These included five stations from southern Mato Grosso and six stations from northern São Paulo (BDMEP, 2013b). Sugarcane-growing regions were identified from CANASAT (http://www.dsr.inpe.br/laf/canasat/), a Landsat-based map of sugarcane cultivation maintained by the Brazilian Instituto Nacional de Pesquisas Espaciais.

5.2.2 Decreased cost of agricultural inputs

Alternatively, if yield increases are not possible due to biophysical limits, biochar use in combination with other organic amendments may help to offset fertilizer use, or to increase fertilizer use efficiency. Previous studies have found that although biochar alone had no effect on plant productivity or nutrient content, biochar in combination with mineral fertilizer or an organic amendment such as compost had a positive effect on fertilizer use efficiency (Chen et al.,

2010; Steiner et al., 2008). It should be noted that, in this thesis, filtercake biochar that had been allowed to age several months in the soil was not effective in reducing NO_3^- losses from cultivated soil (**Chapter 3**), as has been frequently reported for fresh chars (Angst et al., 2013; Knowles and Robinson, 2011; Yao et al., 2012; Zheng et al., 2013). This has been attributed to surface oxidation of biochar in soil (Cheng et al., 2008), leading to an increasingly negative surface charge and consequently lower NO_3^- sorption. However, other studies have found positive effects of biochar on total soil nitrogen, soil organic nitrogen, and NH_4^+, which may not be affected by ageing in the same way (Clough et al., 2013).

Due to its powerful effects on pH, biochar may also help to reduce lime usage on acidic Cerrado soils. Lime has not only a high financial cost, but also a quantifiable environmental cost due to CO_2 emissions that occur during production (calcining), transport/application to sugarcane fields (fossil fuel use), and from its neutralizing action in the soil itself (Macedo et al., 2008). On a 10,000-ha farm in Brazil, lime application at a typical application rate of 2 Mg ha^{-1} would require 20,000 Mg of lime in a single application. Based on an estimated emission factor of 0.477 Mg CO_2-eq per Mg lime used in sugarcane fields (Macedo et al., 2008), a single application to a farm of this size would account for 9,540 Mg CO_2-eq per year removed from geological storage and would cost approximately $1.2 million USD (at a unit cost of $60 USD Mg^{-1} lime, Anonymous, 2012), with re-application every 4–5 years for sugarcane. This thesis does not present sufficient information to calculate the amount of biochar necessary to replace part or all of the lime required on the plantation; however, the liming potential of filtercake biochar is likely to be high, depending on the soil it is applied to, given that a 5% d.w. treatment increased pH by more than

1 unit (**Section 2.3.2**). Even so, it would be necessary to determine whether the amount required for liming would still be within the optimal application range for plant productivity.

Biochar may also positively or negatively influence the leaching and persistence of pesticides used in sugarcane cultivation. In one study, the inclusion of biochar reduced glyphosate leaching from potted ryegrass, but did not influence the degradation of the compound in the soil (Hagner et al., 2013). Biochar has also shown the capacity to strongly sorb triazine pesticides (*e.g.*, simazine and atrazine) as well as organophosphorus pesticides (Uchimiya et al., 2012; Zheng et al., 2010). Provided that sorption does not interfere with the biocidal activity of these compounds, biochar may help to reduce the amount (and cost) of pesticide applied by decreasing leaching losses, while also reducing the risk of unintended ecological effects.

5.2.3 Potential for additional bioenergy generation

Typical sugar/bioethanol facilities in Brazil currently produce only two energy products: bioethanol and electricity, the excess of which is sold back into the grid. However, it is widely recognized that Brazil's bagasse resources are not being exploited to their fullest potential,[11] and there is considerable research interest in further diversifying the product portfolios of these facilities to create more robust, generalized 'biomass energy' plants. Current lines of research include improving electrical output for currently used cogeneration systems (Guerra et al., 2014), integrating bioethanol and soybean biodiesel production (Souza and Seabra, 2013), and finally shifting bagasse resources altogether from cogeneration to the production of second-generation

[11] At the national scale, only ~13% of bagasse produced is used for electricity generation (EPE, 2013). The remainder of the bagasse biomass is burned for steam-only generation, mostly in ethanol and sugar production, which reflects the fact that many existing bioethanol distilleries do not have sufficient cogeneration capacity to meet their own needs and sell to the grid (Hofsetz and Silva, 2012).

(2G) or lignocellulosic bioethanol (Dias et al., 2011). Recent work has suggested that committing bagasse resources to 2G ethanol rather than cogeneration might be the most profitable and robust option given the relative volatility of ethanol and biomass electricity prices (Furlan et al., 2013).

In this context, filtercake pyrolysis presents yet another option for additional biomass energy production, with numerous potential benefits and perhaps also relieving demand for the already fully utilized bagasse resource. These benefits include the soil quality and potential productivity improvements addressed in this thesis, as well as the production of additional saleable bioenergy products, without exorbitant cost. These additional energy products include syn-gas, biochar, and bio-oil. Syn-gas is composed of CH_4, CO, and H_2, and is burned during pyrolysis to maintain the pyrolysis reactor temperature, to produce steam, and to generate electricity (Quirk et al., 2012). Biochar itself is a saleable energy product with an energy density roughly equivalent to that of coal and can be used in the same way with retrofitting (de Ruiter et al., 2014). Finally, a small amount of bio-oil may also be produced, although the process used in this thesis (conventional or slow pyrolysis) maximizes the production of biochar (~35% weight of dry feedstock) and produces syn-gas, but very little bio-oil (Demirbas and Arin, 2002).[12]

For a 'small' 10,000-ha plantation producing at the state average of 68 Mg sugarcane ha^{-1} yr^{-1}, we would expect an annual production of 24,000 Mg of wet or 7,000 Mg dry filtercake.[13] Based on work examining electricity generation via pyrolysis with combustion of syn-gas in a gas

[12] Alternatively, if the plantation and market demand for biochar is weak, it may be more profitable to optimize pyrolysis for bio-oil, which has much higher heating value (Brown et al., 2011).
[13] These calculations assume that filtercake is produced at a rate of ~35 kg Mg $cane^{-1}$ and that it is 70% water (George et al., 2010; Prado et al., 2013).

engine using dry bagasse as a feedstock (0.5 MWh Mg dried bagasse^{-1}, Quirk et al., 2012), this dried biomass represents potential electricity production of 3,500 MWh y^{-1} as well as 2,450 Mg of biochar y^{-1}. This would allow the plant to amend 53 ha of soil y^{-1} at a more moderate dose of 3%,[14] which would contribute 907 Mg of C to the soil if filtercake were the only feedstock used to make biochar. It may also be strategic to initially target biochar application to vinasse-receiving areas, which would allow these areas at greater risk of leaching to be amended in a much shorter timeframe. Alternatively, the biochar could be sold as a substitute for charcoal in Brazil's pig iron industry, which for the most part still relies on environmentally devastating traditional charcoal production that is supplied in part by illegal logging (Muylaert et al., 1999). However, it should be noted that previous modeling studies found that biochar use resulted in 2–5 fold greater GHG reductions when incorporated into the soil than when combusted as biocoal (Gaunt and Lehmann, 2008), owing to reductions in fertilizer usage and displaced fossil fuel emissions.

Filtercake pyrolysis would serve to reduce the use of bagasse for cogeneration. Assuming that the distillery itself would use 14 kWh per Mg of cane processed (Macedo et al., 2008),[15] the exploitation of filtercake alone for power generation would cover ~37% of the plant's total yearly electricity needs (9,520 MWh), freeing what is assumed to be an equivalent mass of bagasse for more profitable purposes (*e.g.*, 2G ethanol production). If filtercake pyrolysis and cogeneration were used in addition to existing bagasse-driven steam and power generation (*i.e.*, filtercake electricity is surplus), electricity solely from filtercake biomass would amount to

[14] Assuming an incorporation depth of 10 cm and a soil bulk density of 1.58 g cm^{-3}, as determined in the field.
[15] This figures refers only to the plant's electricity needs for non-heating purposes. All heat necessary for fermentation and cooking/crystallization is derived from steam, which is produced in excess when bagasse is burned.

$197,000 USD yr^{-1} when sold back into the grid, based on the 2013 auction price for Brazilian bioelectricity ($56.60 USD MWh^{-1})[16]. Less the equipment and heating costs associated with pyrolysis (approximately $14.40 USD MWh^{-1}), there may still remain some incentive for bioethanol distilleries to pursue filtercake pyrolysis and cogeneration. The establishment of such a separate small-scale system is certainly feasible, given the commercialization of pyrolysis–cogeneration systems such as the PyroChar 4000 (Pacific Pyrolysis, Australia), which processes up to 96 Mg of wet feedstock day^{-1}. This system can also be used with multiple feedstocks, such that the plant could continue to benefit from cogeneration using other biomass sources (*e.g.*, corn stover, animal waste, straw) even after the sugarcane harvest has ended. Given the many other options and high costs of technological investment in Brazilian biomass energy, it would be easy to overlook an opportunity like filtercake pyrolysis. However, whether filtercake pyrolysis were used to supplement bagasse cogeneration or established separately from it, exploitation of this resource could have economic benefits at the plant level.

5.2.4 Biochar credits as a (far) future offsetting tool

Biochar has been advocated as a potential soil C sequestration mechanism. Leading academics on this subject suggest that biochar use in soil could potentially avoid 130 Pg CO_2-C equivalents over the next century (Woolf et al., 2010). Although committing biomass to this use would detract from biomass energy development, the authors reported that biochar soil amendment had a 22–27% larger mitigation impact than combusting the same biomass for energy. For this reason, biochar has been very actively promoted as wedge toward climate stabilization, with

[16] http://www.renewableenergyworld.com/rea/companies/green-power-conferences-3234/news/article/2013/09/brazils-a-5-power-auction-contracts-nearly-us9bn-biomass-hydro-power

additional economic benefits through the sale of C credits derived from biochar C sequestration, particularly in biofuels (Mathews, 2008). However, a survey of biochar 'experts'[17] in 2009 identified major remaining uncertainties regarding biochar use for climate mitigation through soil sequestration. Concern focused around the permanence of biochar C, effects on crop yields and GHG emissions, and effects on sustainable development, particularly in developing countries (Shackley et al., 2011). This is concerning given that, in the model used by Woolf et al. (2010), the enhanced climate mitigation impact of biochar over bioenergy use was dependent on assumed increases in crop yields and reductions in soil GHG reductions. Thus, as Woolf et al. (2010) likewise acknowledge, a great deal of work remains to be done regarding some of the less certain and more impactful assumptions regarding biochar effects in soil.

This level of uncertainty may stem from the fact that the vast majority of biochar studies are short-term (<1 year), laboratory-based incubations. Soil incubations frequently report calculated mean residence times (MRTs) on the scale of centuries to millennia for various chars (Cheng et al., 2008; Major et al., 2010a; Novak et al., 2010; Zimmerman, 2010), which under the Kyoto Protocol is considered suitably permanent for sequestration. However, field studies have reported unexpectedly large biochar losses (20–50%) *not* attributable to mineralization, but perhaps due to surface erosion (Husk, 2009; Major et al., 2010a). In addition, the same biochar may mineralize much more quickly in certain soil types (Fang et al., 2013), which may not be adequately represented in laboratory studies. This lack of long-term field studies and the observed variability among char and soil types makes it difficult to offer a certified emissions reduction within an

[17] Experts included primarily academics with partial time commitments to biochar research, business people, policy makers, and workers within non-governmental organizations.

acceptable range of error. In addition to issues with permanence, there may also be valid concern regarding the additionality of sequestered biochar C. Given the significant effects on productivity, and the ability to produce biochar cheaply on a small scale, it is difficult to argue that farmers would not or could not have applied biochar without the cash incentive offered through the sale of C credits. Furthermore, given that biochar amendment has a lesser or null effect on soil parameters or productivity in high-quality soils (Jeffery et al., 2011), farmers in some regions may have insufficient incentive to engage in this practice. Finally, some have raised concern that soil C sequestration credits through biochar will allow large enterprises to accrue much greater benefit than small producers through economies of scale, and that these big players would initiate land grabs that will displace farmers and food production and/or promote habitat loss (*i.e.*, leakage) (Leach et al., 2012). These social justice issues related with the use of biochar, as well as its unintended negative environmental impacts, are very poorly represented in the literature at present. Such concerns may explain why, despite persistent lobbying by the International Biochar Initiative, the use of biochar as a C-sequestering soil amendment has been repeatedly suggested but ultimately denied inclusion in the United Nations Framework Convention on Climate Change (UNFCCC) (Reed, 2011).

Despite these objections, however, academic institutions, governments, and private companies have partnered in the development of protocols for biochar-based soil C credits. These include the Alberta Biochar Initiative in Canada[18] and Australia's Carbon Farming Initiative,[19] which seek to develop highly specific protocols to produce and apply biochar from an appropriate

[18] http://albertabiochar.ca
[19] http://www.climatechange.gov.au/reducing-carbon/carbon-farming-initiative

feedstock and to monitor soil C after application. Although these developments are promising, the work requires significant, long-term, site-specific research investment from interested parties. In addition to research costs, pyrolysis itself has costs, even when biomass is cheap and plentiful; it is estimated that, in order for pyrolysis–cogeneration with soil biochar amendment to become viable, the price of C would have to rise to at least \$37 USD Mg^{-1} CO_2 (Lehmann, 2007). Thus, because of the high research costs, institutional barriers, and the continuing low price of C (\$4–5 USD Mg^{-1} CO_2)[20], biochar C credits from soil sequestration are not likely to be available to Brazilian sugarcane producers over the short-term. A more viable approach than soil C sequestration may be to market biochar as a replacement material for unsustainable charcoal or coal in Brazil's massive pig iron industry (Fick et al., 2013).

5.3 Future research directions

The work presented here represents an initial assessment of the potential utility of filtercake biochar in sugarcane cultivation, relative to the raw residue amendment. In addition to the unknowns regarding the feasibility and profitability of using biochar in bioethanol production (as discussed in **Section 5.2**), there are a number of specific research problems that follow directly from this work.

5.3.1 How does filtercake biochar affect soil microbial communities?

The immediate next step is to complement the physicochemical characterization presented here with a more in-depth study of biological effects. This thesis demonstrated that filtercake biochar has myriad effects on the soil environment, including pH, CEC, water-holding capacity, C and

[20] http://www.pointcarbon.com

nutrient availability, and the sorption of DOC and other substances. As such, the use of filtercake biochar is likely to lead to functionally significant changes in the soil microbial community that could effect both sugarcane productivity as well as GHG accounting and lifecycle analysis for sugarcane bioethanol. In a previous study, Anderson et al. (2011) used terminal restriction fragment length polymorphism (TRFLP) profiling to demonstrate that pine biochar elicited a number of significant changes on the abundances of several bacterial families over time after 12 weeks of incubation. These included increases in the abundance of groups involved in nitrogen fixation and dentrification (which may support decreased N_2O emissions), methane oxidation (decreased CH_4 emissions), cycling of recalcitrant C like lignin, and the solubilization of phosphate (increased productivity). However, in some cases, biochar may also have negative impacts on soil microbiota. For instance, Dempster et al. (2011) found that the inclusion of biochar caused a dose-dependent decrease in microbial biomass and SOM mineralization, which was attributed to unidentified toxic volatile substances. Thus, profiling of the bacterial community in response to filtercake biochar may be necessary to explain or predict the effects of this amendment on soil quality and function (Ameloot et al., 2013).

However, a better understanding of effects on microbial communities and C turnover require reliable tools. Here, we attempted to use a very direct approach by analyzing enzyme activity as an indicator of microbial activity and soil microbial biomass to complement the soil carbon and $\delta^{13}C$ analyses, which were strongly impacted by the presence of filtercake biochar; it may be possible to optimize these assays, as described in **Appendix F**. However, the presence of biochar may also interfere with more sophisticated methods commonly used to examine microbial diversity. For example, DNA isolation has proven problematic in the past (Lehmann et al., 2011)

due to low extraction efficiencies. It is currently unknown whether biochar interferes with the isolation of phospholipid fatty acids (PLFA), although the data in **Chapter 3** suggest that filtercake biochar may adsorb higher-molecular-weight, complex compounds through hydrophobic interactions.

5.3.2 How does filtercake biochar affect sugarcane growth and productivity?

In addition to examining effects on the soil microbiome, a next step is to examine effects on sugarcane growth and productivity in pot and plot experiments. Although previous work supports positive effects on sugarcane productivity (**Section 5.2.1**), feedstock type rather than pyrolysis temperature, soil type, or application rate, is the single most important factor in determining effects on plant growth (Rajkovich et al., 2011). Thus, biochar-specific characterization must be carried out for a given application.

To begin, germination assays are recommended as a means to detect adverse effects due to the presence of phytotoxic bio-oils and volatile compounds clinging to the surface of the char (Major, 2009). Previous work has shown both positive and negative impacts on wheat germination using a variety of biochars, with negative effects predominantly observed at higher amendment rates ($\geq$ 20 Mg ha^{-1}) (Solaiman et al., 2011). Furthermore, Deenik et al. (2010) showed that volatile matter content was associated with decreased growth in both lettuce and corn, which was attributed to a sudden nitrogen deficiency elicited by rapid microbial biomass growth. Furthermore, it has been noted that relatively low doses of some biochars are sufficient to induce positive effects (Novak et al., 2009a; Rajkovich et al., 2011), whereas exceeding these doses may have no additional benefit or may inhibit plant growth (Rajkovich et al., 2011). In

addition, the incorporation of biochar to the soil decreases soil albedo and increased soil surface temperatures (Genesio et al., 2012), which may affect the growth or survival of young plants. In this project, the addition of 5% biochar to cultivated or forests soils did not decrease albedo significantly (**Appendix G)**; however, albedo measurements were taken under dry conditions and the formation of a white fungus or precipitate on the surface of the soil may have affected measurements. Regardless, given that soil surface temperatures in this region can reach 45°C on hot days (personal experience), the effect of biochar on soil albedo and heat fluxes merit further attention.

5.3.3 What information is necessary to implement biochar in bioethanol production?

As has already been discussed, a great deal of additional information is necessary to determine whether or not biochar implementation in Brazilian bioethanol production would be feasible. To develop a comprehensive balance sheet for biochar integration, it is necessary to determine the costs of installing, operating (labour), and maintaining a pyrolysis unit of the appropriate scale, expressed as the price per Mg of biochar produced. Regarding biochar benefits, it would be helpful to quantify increases in sugarcane productivity (through plot or modeling studies) and the savings due to decreased fertilizer and lime use. Further work is also needed to determine the effect of biochar on the efficacy and application rates of pesticides. Finally, biochar use may require additional expenditures on specialized equipment or application techniques to safely apply biochar to the field. Conventional methods used for spreading pelletized soil amendments can result in very large losses of 25–50% biochar applied due to surface erosion (Husk, 2009; Major et al., 2010b). Because erosion of fine-particulate black C may present health risks similar to those observed in charcoal workers (Kato et al., 2005), one option may be to utilize sub-

surface injection technology for liquid fertilizer applications. A slurry made of out of filtercake biochar and vinasse would add stable C, nutrients, and water to soils, although would certainly be more labour-intensive than current vinasse application techniques (high-pressure sprinklers). Answers to these and many other questions are necessary to fully appreciate the utility of biochar in sugarcane bioethanol production.

5.4 Overall significance

The overall objective of this thesis was to better understand C fluxes due to crop residues in sugarcane cultivation and bioethanol production. Specifically, the aim was to reconsider the role of filtercake, a highly underutilized, under-researched residue that is a major by-product in this massive global industry with an annual production of approximately 73 million tonnes.[21] This thesis took an agronomic approach focusing on the use of filtercake as biochar. Biochar, although primarily researched as a means to increase soil C storage and mitigate climate change, also has very great relevance for agricultural productivity and soil remediation. In addition, the sugarcane industry has great potential, through its abundance of feedstocks and reliance on poor tropical soils, to benefit from biochar technology; such a move could also greatly impact global soil C storage due to the massive spatial extent of this industry (26 million ha in 2012; FAOSTAT, 2012). Thus, this thesis used in-depth chemical characterization and a suite of soil quality assays to assess the most critical agronomic benefits of filtercake biochar.

[21] This assumes that 40 kg filtercake is produced per tonne of cane (Prado et al. 2013) and a global production of 1.8 billion tonnes of sugarcane in 2012 (FAOSTAT 2012).

Although assessing the feasibility of integrating biochar technology with on-plant operations is beyond the scope of this thesis, the information provided herein is the first step to such a goal. The data show, in principle, how filtercake biochar could be used to significantly improve soil quality, in terms of pH, CEC, and nutrient and water availability, and reduce C losses. These are the necessary and useful assays that must be performed before investing significant time and research money on field trials. Furthermore, these data are useful in biophysical modeling of sugarcane growth and productivity, and demonstrate a means through which crop residue management could be manipulated to reduce long-term SOC losses, if not reverse them. Thus, the findings of this thesis offer a strong justification for continuing research into the effect of filtercake on sugarcane productivity and the feasibility and profitability of incorporating biochar technology into current and future bioethanol operations.

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Appendices

Appendix A Data and calculations for an in-field sugarcane carbon budget

A.1 Calculating carbon fixation and allocation to plant parts

Total harvested biomass is calculated from harvested cane (68 Mg cane ha^{-1} for Mato Grosso), assuming that cane makes up only 47% of total sugarcane biomass (Resende et al., 2001):

$$\textit{Total harvested biomass} = (68 \times 100) \div 47 = 145 \text{ Mg biomass ha}^{-1}$$

Total C content of the biomass is calculated assuming a moisture content of 75% and an average C content of 45% dry weight (Resende et al., 2001):

$$\textit{Total C content} = 145 \text{ Mg biomass ha}^{-1} \times 0.25 \times 0.42 = 15.2 \text{ Mg C ha}^{-1}$$

C allocation among plant parts is calculated using carbon allocation ratios published by Resende et al. (2001):

$$C_{cane} = 15.2 \text{ Mg C ha}^{-1} \times 0.47 = 7.1 \text{ Mg C ha}^{-1}$$

$$C_{roots+exudates} = 15.2 \text{ Mg C ha}^{-1} \times 0.22 = 3.3 \text{ Mg C ha}^{-1}$$

$$C_{leaves+flags} = 15.2 \text{ Mg C ha}^{-1} \times 0.30 = 4.6 \text{ Mg C ha}^{-1}$$

However, when accounting for C from leaves and flags left on the field, it is necessary to take into account the continued use of pre-harvest burning in some regions, which reduces this removal by up to 95% (Cerri et al., 2011).

A.2 Calculating carbon contributions of organic wastes

Vinasse contribution was calculated assuming the return of vinasse produced directly from the harvested cane, assuming a production rate of 992 L vinasse Mg cane^{-1} (Macedo et al., 2008), and a vinasse C content of 15,500 mg C L^{-1}, based on a review of the literature (Benke et al., 1998; Casarini et al., 1987; Doelsch et al., 2009; Gloeden et al., 1991; Gonçalves et al., 2009; Ribeiro et al., 2011; Silva et al., 2006):

$$C_{vinasse} = 992 \text{ L vinasse Mg cane}^{-1} \times 68 \text{ Mg cane ha}^{-1} \times 15{,}500 \text{ mg C L}^{-1}$$

$$= 1.05 \times 10^{9} \text{ mg C ha}^{-1} \text{ or } 1.1 \text{ Mg C ha}^{-1} \text{ from vinasse}$$

However, vinasse is often applied at or above the maximum allowable rate of 300 m^3 ha^{-1}:

$$C_{max.\ vinasse} = 300{,}000 \text{ L ha}^{-1} \times 15{,}500 \text{ mg C L}^{-1}$$

$$= 4.7 \times 10^{9} \text{ mg C ha}^{-1} \text{ or } 4.7 \text{ Mg C ha}^{-1}$$

Filtercake C concentration was calculated assuming production of 35 kg filtercake Mg cane^{-1} and a moisture content of 70% (George et al., 2010; Prado et al., 2013) and a C content of 37.2%, based on work done in **Chapter 2** and in the literature (Almeida Júnior et al., 2011; Carmo et al., 2013; Elsayed et al., 2008; Galdos et al., 2009; George et al., 2010; Lisboa et al., 2011; Rasul and Khan, 2008; Silva et al., 2007):

$$C_{filtercake} = 35 \text{ kg Mg cane}^{-1} \times 68 \text{ Mg cane ha}^{-1} \times 0.30 \times 0.372$$

$$= 266 \text{ kg C ha}^{-1} \text{ or } 0.3 \text{ Mg C ha}^{-1}$$

However, some experts recommend the application of up to 100 Mg filtercake ha^{-1}, which would amount to a C application of 37.2 Mg C ha^{-1} (cited in Prado et al., 2013).

Appendix B Photographs of experimental apparatus

B.1 Collecting vinasse from Usina Pantanal

Figure B.1. Vinasse collection from a canal.

Vinasse was collected from the nearest point to the plant that was safe to access. Hot vinasse leaves the plant and is briefly stored in a cement-lined pit before being pumped through underground pipes to the top of a gravity-drained canal system. Vinasse was collected by submerging sampling equipment as close as possible to the main current of the vinasse flowing through the canal.

B.2 Building a benchtop biochar reactor

Figure B.2. Benchtop biochar reactor.

The reactor was constructed out of stainless steel pipe (50 cm long × 15 cm in diameter) fitted with an N_2 purge and closed with a steel plate and C-clamp. The dimensions were dictated by what could fit in the available heat source (a large muffle furnace).

Figure B.3. Assembling the reactor.

The reactor was set inside a large electric muffle furnace. Copper tubing (1/4″) was run through the vent of the muffle to conduct N_2 gas in to the right end of the reactor. A second piece of tube at the other end was used to vent the reactor out of the laboratory. In addition to the gas lines, two type-K thermocouples connected to a Campbell Scientific CR10X datalogger were passed through the muffle vent. One thermocouple was inserted into the middle of the biomass via a port on the left end of the reactor. The other thermocouple was allowed to hang in the center of the furnace itself to track temperature fluctuations due to the muffle.

Figure B.4. Filtercake biochar.

After the pyrolysis program was complete (approximately 3 h), the reactor was allowed to cool overnight with a continuous flow of N_2 gas to prevent combustion. The finished product retained the outward appearance of the original plant biomass, but was fully pyrolyzed upon visual inspection.

B.3 CO_2 efflux assay apparatus

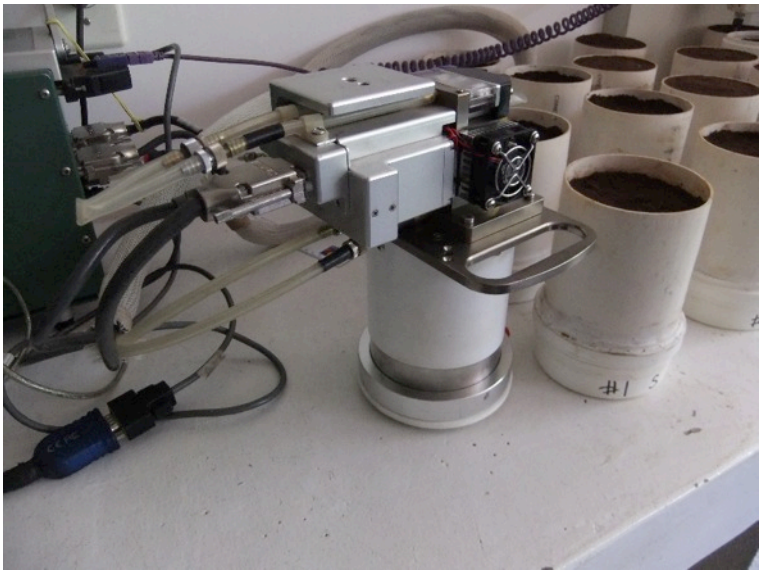

Figure B.5. Modified apparatus used in the CO_2 efflux assays.

CO_2 efflux was monitored using the LI-COR 6400 XT apparatus (LI-COR, Lincoln, NE), which was fitted over the incubation column using a PVC sleeve (not pictured) and a foam rubber gasket to form a gas-tight seal. This allowed CO_2 fluxes to be monitored without first disturbing the soil through movement; instead, the LI-COR could be carefully set over the top of each column. Temperature of the soil was monitored by inserting the temperature probe in a fourth column reserved for this purpose. On day 8 and day 13, water/vinasse was added only after CO_2 efflux measurements had been collected.

B.4 Leaching column apparatus

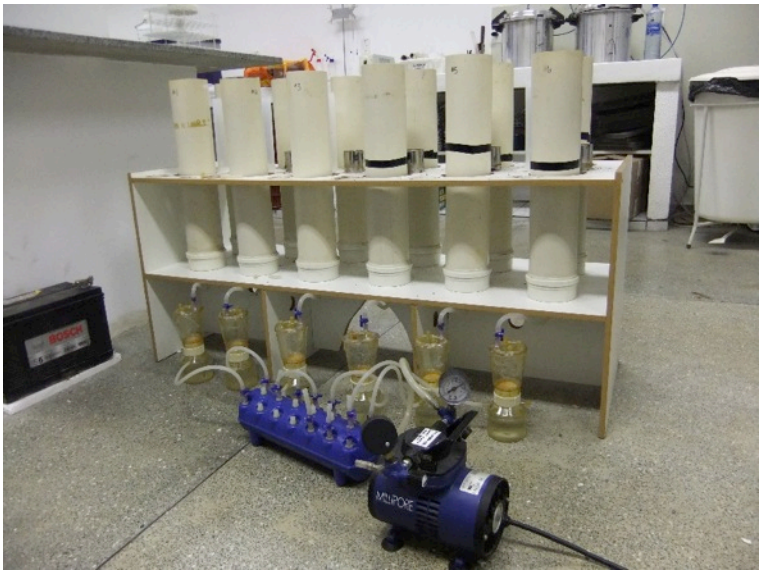

Figure B.6. Leaching column apparatus.

Twelve PVC leaching were mounted in a custom-made stand. Silicone Tygon Tubing (1/4″) and a small plastic valve were used to connect the outlet of the column to the top of the sample collector, a vacuum filtration unit (Millipore Corp., MA). All filtration units were connected to a single vacuum manifold, such that equal suction was applied to all, but could be controlled individually via the valve at the top of the flask. Suction (–30 kPa) was applied using a Millipore vacuum pump.

B.5 Microbial response incubations

Figure B.7. Soil incubation for microbial response analyses.

Soil used to analyze enzyme activities, microbial biomass, and $\delta^{13}C$ ratios were incubated in the dark for 90 days in plastic pots with perforated lids, as pictured. A CR10X data logger powered by a 9V battery (right) with a thermocouple was used to monitor temperature in this enclosed space.

Appendix C Selection of production parameters for filtercake biochar

The biochar used in this study was produced via conventional or slow pyrolysis (275–675°C for minutes to hours), which favours the production of biochar rather than bio-oil, as in fast pyrolysis (575–975°C for a few seconds) (Demirbas and Arin, 2002). Although different feedstocks will produce biochars with different properties, certain aspects of the slow pyrolysis program have a large influence over the final char characteristics. For example, for bagasse biochar, increasing the highest heating temperature (HTT) increases final % C, increases final surface area, and decreases CEC (Kameyama et al., 2012; Novak et al., 2009b). Increasing HTT greater than 600°C may also shift the chemical properties of a given char such that they become nitrate-retaining rather than nitrate releasing (Yao et al., 2011). In addition, increasing the residence time (duration of pyrolysis) also decreases the % labile C (Cross and Sohi, 2011), which is desirable in biochars that are meant to be relatively more stable in the soil. These trends generally (but do not always) hold true for a variety of feedstocks (Gaskin et al., 2008; Singh et al., 2010; Yao et al., 2012).

In this project, the key parameters of concern are the ability to absorb and retain water and nutrients, which are directly related to surface area and porosity, and the priming potential due to the presence of labile C. Based on other sugarcane (bagasse) chars presented in the literature (Chen et al., 2010; Inyang et al., 2010; Kameyama et al., 2012) and the general trends described above, biochar in this study was produced by heating filtercake to 575°C for 3 h at a rate of 5°C min^{-1}.

Appendix D Rationale for biochar application rates

Four experimental biochar application rates were selected based on 1) the mean application rates of these residues to cane fields nearest to the distillery, 2) the average percent yield after pyrolysis (approx. 35%), 3) and the effect of annual application. If approximately 5 t of dry filtercake are applied per hectare (Macedo et al., 2008), this corresponds to a base rate of 1.75 Mg biochar ha^{-1} (or a 0.07% w/w addition, based on a cultivated soil depth of 10 cm and a bulk density of 1.5 g/cm^3). This application is very low and unlikely to have an immediate effect on soil quality. However, given that filtercake disposal is a perennial problem and is applied repeatedly to the same field over many years, application rates were selected to reflect biochar accumulation over decades (**Table C.1**), assuming minimal loss in this time frame for high-temperature biochars (Zimmerman, 2010). This broad range is both realistic and spans the treatment doses applied in the literature (Laird et al., 2010; Novak et al., 2009a).

Time (years)	*10*	*20*	*40*
Accumulated biochar (Mg ha^{-1})	17.5	35	70
Experimental dose (% w/w)	1.2	2.3	4.7

Table D.1. Experimental biochar doses and their field application equivalents.

Appendix E Effects of residue treatments on post-leaching soil quality

Soil collected before column packing and after leaching was analyzed for pH, CEC_e, total organic carbon (TOC), and several macronutrients, as described in **Chapter 3**. Biochar had an alkalinizing effect on soil pH, both before leaching (pre-S *vs*. pre-SB) and after leaching with a total of ~7300 mL of water over several months (S *vs*. SB, **Table E.1**). Likewise, 5% biochar inclusion greatly increased the initial soil CEC_e (pre-S *vs*. pre-SB) and retained part of this effect post-leaching (pre-SB *vs*. SB). Regarding total organic C, biochar inclusion resulted in a small but significant increase in total organic C at a dose of 5% (pre-S, 1.48 ± 0.06%; pre-SB, 2.65 ± 0.03 %; $p < 0.001$); after leaching, TOC levels remained elevated in the SVB and SB treatments, indicating that the ground biochar remained in place in the 0–10 cm layer. In contrast, comparison of the S *vs.* SV treatments (post-leaching) revealed that vinasse application had no effect on pH, CEC_e, or % C, and did not result in %C increases compared to the pre-leaching soil. No significant effects on soil %N were observed in pre- and post-leaching samples.

In terms of soil macronutrients, the addition of biochar before leaching increased the availability P, K, Ca, and Mg in pre-leaching soil samples (pre-S *vs.* pre-SB, **Table E.1**). Post-leaching, which integrates the effects of this experiment and all previous water/vinasse inputs to the columns, soil containing biochar (SVB and SB) lost nutrients relative to the pre-leaching control (SB), but retained a significant nutrient benefit compared to the S and SV columns. The effect of cumulative vinasse application (1 L) also caused slight increases in P and K availability compared to the pre-leaching S control, whereas Ca and Mg were lost from both the S and SV columns. However, the SVB treatment receiving both vinasse and biochar showed enhanced retention of K compared to all other treatments.

Table E.1. Additional soil quality data for pre- and post-leaching soils

Soil pH, effective cation exchange capacity (CEC_e), total C, and macronutrients in unleached soils and in leached soils treated with biochar and/or vinasse. Data were analyzed via one-way analysis of variance (ANOVA) followed by *post hoc* Tukey test for comparisons among means. Data marked with an asterisk were log-transformed before analysis; all data are means ± SE (n = 3) and significance was set at $p < 0.05$.

Treatments	***pH (H_2O)***	***CEC_e ($cmol_c/dm^3$)***	***Total C (%)***	***Total N (%)***	***Extractable Nutrients***			
					P * (mg/dm^3)	**K (mg/dm^3)**	**Ca ($cmol_c/dm^3$)**	**Mg* ($cmol_c/dm^3$)**
Pre-leaching								
Pre-S	6.13 ± 0.03 *a*	7.97 ± 0.24 *a*	1.48 ± 0.06 *a*	0.07 ± 0.01 *a*	[#]1.50 ± 0.10 *a*	46.67 ± 0.67 *a*	3.13 ± 0.08 *a*	1.49 ± 0.11 *a*
Pre-SB	7.40 ± 0.03 *b*	11.63 ± 0.38 *b*	2.65 ± 0.03 *b*	0.08 ± 0.01 *a*	151.57 ± 17.81 *b*	243.00 ± 10.79 *b*	6.66 ± 0.27 *b*	3.52 ± 0.15 *b*
Post-leaching								
S	5.83 ± 0.03 *c*	7.50 ± 0.32 *a*	1.37 ± 0.08 *a*	0.03 ± 0.01 *a*	[§]2.27 ± 0.33 *ac*	[§]78.00 ± 2.31 *c*	2.97 ± 0.10 *a*	1.40 ± 0.05 *a*
SV	5.80 ± 0.06 *c*	7.23 ± 0.15 *a*	1.42 ± 0.05 *a*	0.03 ± 0.01 *a*	2.77 ± 0.15 *c*	101.67 ± 1.33 *c*	2.78 ± 0.06 *a*	1.40 ± 0.01 *a*
SVB	8.10 ± 0.00 *d*	8.50 ± 0.46 *c*	2.54 ± 0.53 *b*	0.07 ± 0.03 *a*	142.40 ± 7.17 *b*	192.67 ± 4.70 *d*	4.99 ± 0.11 *c*	2.75 ± 0.23 *b*
SB	8.23 ± 0.03 *d*	8.53 ± 0.23 *c*	2.93 ± 0.28 *b*	0.08 ± 0.02 *a*	139.93 ± 3.90 *b*	159.00 ± 4.36 *e*	4.84 ± 0.28 *c*	2.97 ± 0.15 *b*

[#] Missing one observation.

[§] The increase in K in post-S columns relative to pre-S is the result of an error in the pre-experimental period in which a small amount (< 100 mL) of vinasse was applied. After this occurred, all columns were flushed extensively with water until parameters (pH, DOC, NO_3^-, turbidity, *etc.*) returned to baseline. At the time of the experiment described in **Section 3.3.1**, no evidence of this occurrence was observed (*i.e.,* the S and SV treatments are not significantly different at the beginning of the experiment for any parameter). However, given the extremely high K+ concentration of most vinasse, it is not surprising that the experimental error was evident for only this parameter, even after extensive washing of the column

These data, in combination with the C data discussed in **Section 3.3.1**, show that the addition of biochar has lasting impacts on soil quality parameters, even after several intensive leaching events. In contrast, vinasse had no effect on post-leaching soil quality except for small increases in available P and K, in which vinasse is enriched.

Appendix F Filtercake biochar hinders enzyme and microbial biomass assays

F.1 Introduction

Due to its effects on soil physical and chemical conditions, biochar is in most cases expected to impact soil biota. However, it has been noted by previous authors that many of the methods commonly used to examine biological impacts could be affected by the presence of highly adsorbent charcoal (Bailey et al., 2011; Luo et al., 2013; Thies and Rillig, 2009). In enzyme assays, for example, biochar may bind either the substrate, the reaction product, and/or the enzyme participating in the reaction, leading to an apparent suppression of activity. In a soil-free assay, Bailey et al. (2011) found that pre-exposing the substrate to switchgrass biochar moderately to strongly decreased apparent enzyme activity for β-glucosidase, lipase and leucine aminopeptidase, whereas pre-exposure of the purified enzyme prior to the assay with clean substrate had a lesser or no effect. Alternatively, biochar may increase apparent activity. In the same study, Baily et al. (2011) found that pre-exposure of the purified enzyme or substrate prior to the assay strongly increased apparent activity, which the author attributed to the release of an allosteric upregulator. Another potential mechanism for increased apparent activity is the concentration of substrates and enzymes on charged surfaces in a way that facilitates catalysis. Furthermore, the same assay carried out in different soils showed highly variable results (*i.e.*, apparent increase in activity in one soil type, and a decrease or no effect in others). For biological assays that require extraction of an analyte (*e.g.*, chloroform fumigation–extraction or nucleotide isolation for molecular assays), the presence of an adsorbent may simply prevent isolation and detection of the analyte. Previous work has shown that the presence of biochar

significantly decreases the extraction efficiency of DOC from amended soils (Durenkamp et al., 2010).

Despite these methodological issues, however, several studies have successfully assayed enzyme activity, having first verified that the presence of biochar did not interfere with the assay. Paz-Ferreiro et al. (2011) showed that although sewage sludge biochar (surface area, 32 m^{-2} g^{-1}) did adsorb the reaction product *para*-nitrophenol (*p*-NP) from the assay solution, this did not occur to a greater degree than can be expected in soils showing normal variation in "natural" adsorbents (*i.e.*, humic material and clay minerals). Lammirato et al. (2011) showed that although chestnut wood biochar (surface area, 2 m^{-2} g^{-1}) did indeed sorb up to 97% of β-glucosidase in solution (with no effect on substrate availability), sorption caused only a 30% decrease enzyme activity, which was considered minor by the authors. In contrast, the presence of activated C (surface area, 900 m^{-2} g^{-1}) completely eliminated enzyme activity. Durenkamp et al. (2010) likewise reported that although biochar inhibited C extraction for biomass determination, the overall effect on assay outcome was not significant. Thus, the magnitude of interference in a given assay is dependent on the type of char, as well as potentially the soil type.

Because of these soil- and biochar-dependent effects, and because filtercake biochar was found to significantly reduce DOC leaching in **Chapter 3**, preliminary assays were carried out to determine whether the presence of filtercake biochar affected the reliable determine of enzyme activity or microbial biomass in an amended forest soil. The first enzyme analyzed was β-glucosidase, which is the rate-limiting enzyme in the breakdown of cellulose to glucose and thus reflects the metabolism of relatively labile C. The second "enzyme" analyzed was

polyphenoloxidase (PPO), which in fact refers to a general class of enzymes that use oxygen to oxidize phenols and are thought to be important in lignin degradation and humification (*i.e.,* processing of much more recalcitrant C) (Sinsabaugh, 2010). These assays, along with microbial biomass, were examined because of their primary importance in understanding the biotic impacts of biochar amendment, and specifically because we wished to use them in the interpretation of CO_2 efflux and C utilization data gathered in **Chapter 4**.

F.2 Methods

Soil incubations

Soil from a forested riparian area was collected from the field and transported on ice to the laboratory. This soil was sieved at 4 mm, roots were removed by hand, and the soil gently mixed to ensure a uniform composition. Soil was mixed with 0, 2.5, or 5% (dry weight) filtercake biochar that had been ground to < 2 mm. Treatments were then bagged and returned to the refrigerator until analysis. The soil moisture content of each treatment was determined gravimetrically after drying at 105°C overnight.

Enzyme activity: β-glucosidase and polyphenol oxidase

To examine β-glucosidase activity, we scaled down the assay published by Alef and Nannipieri for analysis in a cuvette (1995). Briefly, 0.50 g of moist soil was suspended in 2 mL of modified universal buffer (MUB; 20 mM Tris, 20 mM maleic acid, 15 mM citric acid, 20 mM boric acid, 100 mM NaOH, pH 6.5) and 0.5 mL of substrate (*p*-nitrophenyl-β-D-glucoside, 25 mM). The tubes were capped and thoroughly mixed, and then incubated to 1 h at 37°C in the dark. Reagent

and soil blanks were also prepared. After incubation, 1 mL of $CaCl_2$ and 1 mL of Tris buffer (pH 1) were used to quench the reaction, after which the tubes were centrifuged at 1250 *g* for 5 min to settle organic material. The aqueous phase (1 mL) was removed without disturbing the sediment or organic material, and then diluted in 3 mL of cold Tris buffer (pH 10). Absorbance was measured at 400 nM. Absorbance values were then corrected against the absorbance recorded for the corresponding treatment reagent blank (soil with 0, 2.5 or 5% biochar, but no substrate until after the incubation period had finished).

To examine polyphenol oxidase activity, we used a modified pyrogallol assay developed with reference to Allison and Jastrow (2006), Carney et al. (2007), and Jindo et al. (2012). Briefly, 0.50 g of moist soil was suspended in 0.5 mL of acetate buffer (50 mM $C_2H_3NaO_2$, pH 5.0) and 2.5 mM pyrogallol dissolved in acetate buffer. The tubes were capped and thoroughly mixed, and then incubated for 2 h at 30°C in the dark. Reagent and soil blanks were also prepared. After incubation, the tubes were centrifuged at 1250 × *g* for 5 min and the aqueous phase (1 mL) was transferred to a clean tube on ice in a dark container. Absorbance due to the production of an oxidized reaction product was measured immediately at 405 nM. Differences among treatments were then expressed as corrected absorbance.

Microbial biomass assays: chloroform fumigation–extraction (CFE) and chloroform fumigation–incubation (CFI)

CFE was carried out as described by Vance et al. (1987) and Wu et al. (1990). Briefly, soil samples (25 g) from each treatment (0. 2.5, or 5% d.w. filtercake biochar) were weighed out into

beakers and subjected to chloroform fumigation in an evacuated dessicator for 48 hours. Paired non-fumigated samples (25 g) were extracted immediately with 50 mL of 0.5 M K_2SO_4 for 30 min. This concentration of K_2SO_4 was selected based on previous work showing that the extractant concentration increased C extraction efficiency in biochar-treated samples (Durenkamp et al., 2010). Fumigated and non-fumigated samples were frozen immediately after extraction until analysis. To determine extractable C, samples were thawed, mixed, settled, diluted and acidified, and finally analyzed for non-purgable organic C using a Jena Analytic Multi N/C Analyzer (Jena Analytik, Germany). Microbial biomass was then calculated by dividing the flushable C by a factor of 0.45 (Wu et al., 1990).

For CFI, soil samples (23 g) were first subjected to chloroform fumigation in an evacuated dessicator for 48 hours, while unfumigated samples (25 g) were kept in closed containers in the dark. Next, fumigated samples were inoculated by transferring 1 g of soil from paired unfumigated samples, followed by mixing. Next, samples were transferred into glass jars containing a 20-mL aliquot of 0.5 M NaOH and allowed to incubate in the dark for 10 days. At the end of the incubation, the NaOH aliquot was withdrawn, treated with 1 mL of 1.5 M $BaCl_2$-$2H_2O$ and 1% phenothalein and then titrated with 0.3 M HCl. Microbial biomass was then calculated according to Horwath et al. (1996).

Statistics

Differences among treatments analyzed in triplicate were detected via one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey text. All assays were carried out in triplicate,

with the exception of the 5% biochar treatment in the CFI assay (n = 2). A p-value of 0.05 was considered statistically significant. All statistical analyses were carried out in R using R Commander (Fox, 2005; R Core Team, 2013).

F.3 Results

Enzymes assays

The presence of 2.5 or 5% biochar in forest soil without any incubation resulted in a ~50% decrease in apparent enzyme activity relative to the untreated soil. Notably, this decrease was not dose-dependent, with a roughly equivalent magnitude of effect exerted by both biochar treatments. In contrast, the presence of biochar elicited a dose-dependent increase in apparent enzyme activity in the PPO assay.

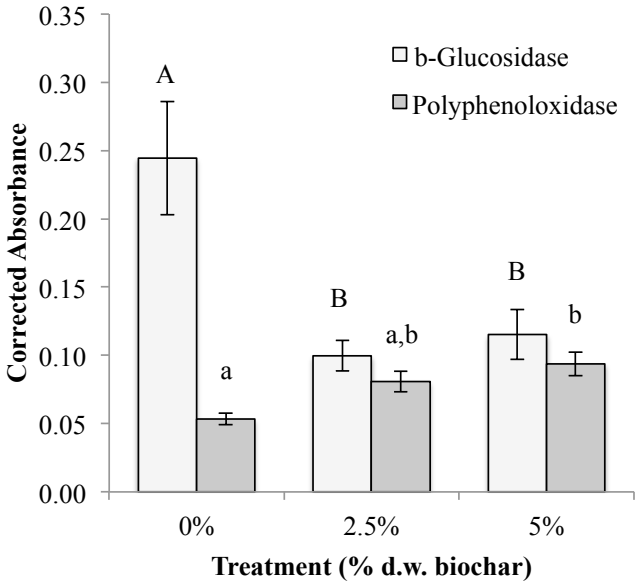

Figure F.1. Apparent enzyme activity in a biochar-amended forest soil.

Soil microbial biomass

As in the enzyme assays, the presence of biochar complicated the determination of soil microbial biomass through CFE, likely due to this product's strong ability to adsorb dissolved organic C and prevent it from being extracted from the soil matrix. In the CFE assay, the presence of 5% biochar in the forest soil resulted in a 65% decrease in apparent microbial biomass when compared to the untreated soil (**Fig. F.2**). In contrast, when analyzing soil via CFI, which estimates biomass through the production of CO_2 rather than soluble C, no differences were observed among treatments, although it should be noted that calculated microbial biomass was much lower overall for CFI *vs.* CFE.

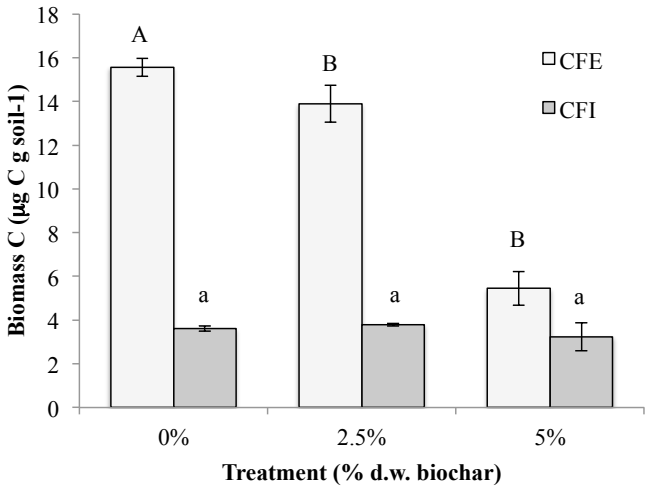

Figure F.2. Comparison of microbial biomass methods in a biochar-amended forest soil.

F.4 Discussion

β-Glucosidase and polyphenol oxidase activity in biochar-amended soil

Previous authors have noted potential issues with the use of standard microbiological methods due to the sorptive capacity of biochar (Thies and Rillig, 2009). In this study, the addition of 5% biochar and immediate analysis resulted in a 50% decrease in absorbance relative to the 0% control, suggesting a biochar-induced decrease in β-glucosidase activity. These results are consistent with those of Bailey et al. (2011) and Jindo et al. (2014), who reported that biochar strongly retained either the enzyme or the reaction product, *p*-NP, from the assay solution, hindering the reaction or its quantification. Thus, in contrast to previous authors who did not find strong interference when including biochar (Lammirato et al., 2011; Paz-Ferreiro et al., 2011; Wu et al., 2012), this data demonstrates that the standard colorimetric protocol for β-glucosidase activity is not appropriate for use with filtercake biochar.

It is not possible to determine from this assay whether the apparent inhibition of β-glucosidase was due to adsorption of the reaction product and/or the enzyme; however, this distinction is very important. If the apparent decrease is due to adsorption of the reaction product, thus preventing colorimetric quanitification, it represents an artifact. Such an artifact could potentially be resolved by using a soil-less assay to generate a "calibration curve" in which increasing concentrations of *p*-NP are briefly incubated with an assay-relevant quantity of biochar (*e.g.*, for the 5% treatment, approximately 20 mg of filtercake biochar in the same assay volume) and then centrifuged at high *g* to remove the biochar and bound product. This would allow the investigator to correct for the amount of *p*-NP retained for a specific dose of a specific biochar.

It may also be possible to reduce biochar-induced artifacts through modifying assay conditions. It has been shown that using an alkaline assay buffer (pH 11), as for alkaline phosphatase activity, results in a much lower degree of *p*-NP retention compared to an acidic buffer (pH 5) (Jindo et al., 2014). This was attributed to a change in the surface charge of biochar in solution and corresponding drop in retention efficiency, and thus altering pH may help to reduce the effects of biochar on reaction product retention. However, in most tropical soils, β-glucosidase activity decreases rapidly at pH values > 7 (Turner, 2010), thus limiting the upper limit to which pH could be adjust without compromising the assay. Furthermore, Bailey et al. (2011) noted that fluorogenic enzyme substrates may be less susceptible to biochar interference, as sorbed, fluorescing substrate can still be quantified (provided that sorption does not induce fluorescence quenching), although other authors have noted strong quenching of sorbed fluorogenic substrates (Lehmann et al., 2011).

However, if the filtercake biochar assayed here caused a decrease in apparent activity by binding and inhibiting the target enzyme, this does not represent an artifact, but rather a functionally relevant effect on the soil ecosystem. However, until a given assay is validated for a specific char, it would be impossible to further investigate these effects.

Regarding the effect of biochar on PPO activity, the inclusion of 2.5 and 5% biochar elicited a linear increase in apparent enzyme activity. A similar increase was also observed in the only other study to use the pyrogallol assay in biochar-treated soils, in which biochar was added to poultry manure compost (Jindo et al., 2012). However, the authors of this previous study present no validation of their assay protocol. One possible mechanism for the observed increase in apparent PPO activity may be an unintended shift in pH. As noted in **Chapter 2**, filtercake biochar is strongly alkalininzing, shifting the pH of this forest soil from 4.0 ± 0.1 to 6.2 ± 0.1 in water. Although this is unlikely to affect assay conditions given the use of a buffer, it is important to note that porous biochar is known to create specialized microenvironments within the soil solution, previously referred to as the 'charsphere' (Luo et al., 2013). Thus, enzyme and substrate coming together in the charsphere may in fact be under different assay conditions. It has previously been noted that the substrate used in this assay, pyrogallol, is quite redox sensitive, and thus oxidation of the substrate may increase if pH increases (Bach et al., 2013). Thus, if filtercake biochar is able to adsorb PPO in the assay solution, it is possible that the alkaline environment of the charsphere could potentiate substrate oxidation and increase apparent activity.

Microbial biomass analyses using filtercake biochar

CFE has become a very common method of determining microbial biomass, due to the relative simplicity and swiftness of this protocol compared to CFI or other methods. However, in **Chapter 3**, we noted that soil columns amended with 5% filtercake biochar showed significantly lower DOC leaching loss compared to unamended columns. The preliminary tests presented here revealed that the presence of 2.5 or 5% biochar significantly reduced the amount of soluble C recoverable through extraction with 0.5 M K_2SO_4. This artificially decreased the apparent biomass contained within the biochar-amended samples, demonstrating that CFE is not appropriate for use with filtercake biochar.

In contrast to our findings, Durenkamp et al. (2010) found that treatment with a hardwood biochar made at 500°C did not significantly decrease C recovery using 0.05 or 0.5 M K_2SO_4 in two out of three soil types tested; however, treatment with activated charcoal did. Other studies have also successfully used CFE in biochar-amended soils without apparent issues with C extraction efficiency (Dempster et al., 2011; Luo et al., 2013; Zavalloni et al., 2011). These results demonstrate the importance of preliminary testing when attempting to characterize the effects of a given soil–biochar mixture.

It is difficult to speculate as to why we observed such a large difference in C extraction efficiency, given that parameters related to the sorption capacity (*i.e.*, surface area, porosity, surface charge) for a given biochar are not routinely reported. Typically, surface area and pore volume for a biochar made from a given feedstock increases with highest heating temperature (Kameyama et al., 2012), thus facilitating absorption, and there is some evidence to suggest that

lower temperature pyrolysis produces biochar with more water-extractable C (Lin et al., 2012). In addition, as biochar ages in the soil, its sorption capacity may change as surface functional groups are oxidized (Liang et al., 2006), or as access to the interior of pores is blocked by organic material (Pignatello et al., 2006). Regardless as to why these results occurred, the data demonstrate that assays must be validated and potentially modified for use with a given biochar, even though some modifications may hinder the comparison of results in the literature.

Appendix G Effect of filtercake biochar on albedo

G.1 Material and methods

Incubation columns used in the CO_2 efflux assays for cultivated (**Chapter 2**) and forest (**Chapter 4**) soils were examined for changes in albedo of the soil surface as a result of vinasse, filtercake, or biochar amendment. The difference between light irradiating and light reflecting from the soil surface was measured using two pyranometers (LI200X; LI-COR Biosciences, Lincoln, NE) connected to a CR10X datalogger (Campbell Scientific, Logan, UT, USA) powered by a 9V battery. These data were graciously provided by Dr. Francisco de Almeida Lobo. For each soil type, treatment means were compared via one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey test, after first performing a Shapiro–Wilk normality test and Levene's test on the median to confirm homoscedasticity. Significance was set at $p < 0.05$.

G.2 Results and discussion

Raw residue or biochar amendment did not significantly affect the proportion of incident light reflected by the soil surface in either soil type, although trends toward decreased albedo were observed in the cultivated soil. These data suggest that, under dry conditions, treatment with 5% d.w. filtercake biochar does not significantly affect energy reflected from the soil surface.

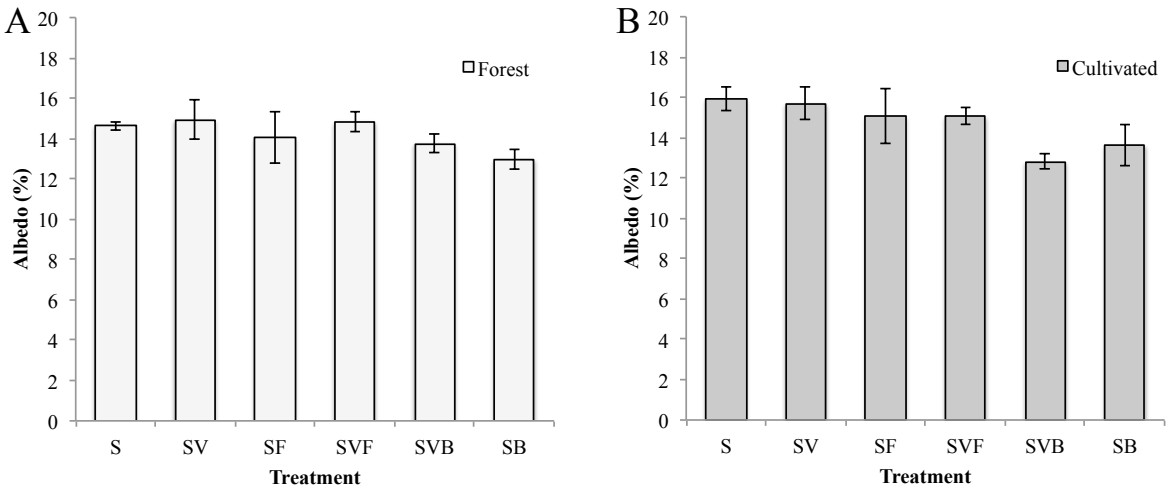

Figure G.1. Effects of 5% d.w. biochar amendment on soil albedo.