**University of Sao Paulo
“Luiz de Queiroz” College of Agriculture**

**The role of biochar on greenhouse gas offsets, improvement of soil
attributes and nutrient use efficiency in tropical soils**

Thalita Fernanda Abbruzzini

Thesis presented to obtain the degree of Doctor in
Science. Area: Soil and Plant Nutrition

**Piracicaba
2015**

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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To my parents, Ricardo and Cleide
To my sister and brother, Thamiris and Ricardo
To my niece, Duda
I DEDICATE

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“A nation that destroys its soil destroys itself.”
Franklin D. Roosevelt

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RESUMO

O papel do biochar nas emissões de gases do efeito estufa, melhoria de atributos do solo e eficiência de uso de nutrientes em solos tropicais

O produto sólido da pirólise, denominado “biochar” (BC) no contexto da melhoria nos atributos do solo como parte do manejo agrícola e ambiental, também tem se destacado na mitigação das mudanças climáticas. O pesquisador investigou os efeitos do BC nos atributos do solo, uso do nitrogênio (*N*) e emissões de GEE. No Cap. 1 comentou-se a origem do BC. No Cap. 2, caracterizou-se o BC de palha de cana-de-açúcar e avaliou-se o potencial de decomposição do *C* do solo, com os tratamentos: (T1) Solo; (T2) BC; (T3) Solo + BC 10 $Mg\ ha^{-1}$; (T4) Solo + BC 20 $Mg\ ha^{-1}$ (T4); e (T5) Solo + BC 50 $Mg\ ha^{-1}$. No Cap. 3, avaliou-se a combinação BC, torta de filtro (TF) e vinhaça (V) em atributos do solo e fluxos de dióxido de carbono (CO_2), metano (CH_4) e óxido nitroso (N_2O) nos tratamentos: (T1) Solo + TF + V; (T2) Solo + TF + V + BC 10 $Mg\ ha^{-1}$; (T3) Solo + TF + V + BC 20 $Mg\ ha^{-1}$; e (T4) Solo + TF + V + BC 50 $Mg\ ha^{-1}$. No Cap. 4 investigou-se a eficiência de uso do *N* num experimento em vasos com trigo usando $NH_4[^{15}N]O_3$ e doses de BC, com os tratamentos: (T1) Solo, com *N*, sem BC; (T2) Solo, com *N*, BC 10 $Mg\ ha^{-1}$; (T3) Solo, com *N*, BC 20 $Mg\ ha^{-1}$; e (T4) Solo, com *N*, BC 50 $Mg\ ha^{-1}$. Os teores de *C* e *N* do BC foram maiores comparado à biomassa. *K*, *Mg* e *P* totais também aumentaram. Os menores fluxos de CO_2 foram do BC. O CO_2 do solo e solo + BC não diferiram. Observou-se maior $CO_2 - C_4$ no primeiro dia de incubação, porém sem diferenças no $CO_2 - C_3$. O BC apresenta características para melhorar atributos do solo e reduzir as emissões de CO_2 . No Cap. 3, *pH*, *P* e bases aumentaram e o Al^{3+} diminuiu com o BC. Os impactos do BC na CTC foram maiores em solo arenoso. O *N* mineral diminuiu com o BC. O CO_2 acumulado no T1 foi maior nos solos arenoso e argiloso comparado ao controle. O T2 e T3 aumentaram o CO_2 acumulado do arenoso relativo ao T1, enquanto T4 e T1 não diferiram. O BC reduziu as emissões de N_2O pelos solos arenoso e argiloso comparado ao T1. O BC combinado à TF e V afetaram *pH*, CTC, *P* e bases do solo arenoso. O BC suprimiu o N_2O de solos com V e TF. No Cap. 4, o BC diminuiu as emissões de N_2O comparado ao fertilizante *N* apenas. T4 teve rendimento de grãos superior ao T1. T2 a T4 apresentaram maior peso de 100 grãos e biomassa aérea. T3 e T4 tiveram maior *N* em grãos. O BC melhora o uso do *N*, a produção de grãos e reduz o N_2O de fertilizante *N*, abrindo perspectivas para a avaliação do BC de palha de cana-de-açúcar na melhoria da qualidade do solo e mitigar das emissões de GEE.

Palavras-chave: Carbono pirogênico; Dióxido de carbono; Manejo do solo; Metano; Método do traçador ^{15}N ; Mudanças climáticas; Óxido nitroso; Sequestro de carbono

ABSTRACT

The role of biochar on greenhouse gas offsets, improvement of soil attributes and nutrient use efficiency in tropical soils

The solid product of pyrolysis, called “biochar” (BC) in the context of improving soil properties as part of agronomic or environmental management, also got into focus as a climate mitigation strategy. The researcher investigated the effects of BC on soil attributes, nitrogen (N) use and GHG emissions. In Chapter 1 the origin of BC was commented. In Chapter 2, BC from sugarcane straw was characterized, and its priming on native SC was evaluated with the treatments: (T1) Soil; (T2) BC; (T3) Soil + BC 10 $Mg\ ha^{-1}$; (T4) Soil + BC 20 $Mg\ ha^{-1}$; and (T5) Soil + BC 50 $Mg\ ha^{-1}$. In Chapter 3, it was evaluated the combination of BC, filter cake (F) and vinasse (V), in relation to soil attributes and carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) emissions. The treatments were: (T1) Soil + FC + V; (T2) Soil + FC + V + BC 10 $Mg\ ha^{-1}$; (T3) Soil + FC + V + BC 20 $Mg\ ha^{-1}$; and (T4) Soil + FC + V + BC 50 $Mg\ ha^{-1}$. In Chapter 4, the nitrogen (N) use efficiency was investigated in a pot trial under wheat using $NH_4\ [^{15}N]O_3$ and rates of BC, with the treatments: (T1) Soil, with N, no BC; (T2) Soil, with N, BC 10 $Mg\ ha^{-1}$; (T3) Soil, with N, BC 20 $Mg\ ha^{-1}$; and (T4) Soil, with N, BC 50 $Mg\ ha^{-1}$. BC had C and N contents higher compared to the feedstock. Total K, Mg and P also increased. The lowest CO_2 fluxes were for BC, and CO_2 from soil and soil + BC did not differ. The highest $CO_2 - C_4$ was in the first day, and there were no differences in the $CO_2 - C_3$. The BC presents characteristics to improve soil attributes. BC stability is an opportunity to reduce CO_2 emissions. In Chapter 3, soil pH, P and base contents increased and Al^{3+} decreased with BC to sandy soil. Impacts of BC on the CEC were higher in sandy soil. Mineral N decreased with BC. Cumulative CO_2 in T1 were higher in sandy and clayey soils than the control. T2 and T3 in sandy soil increased CO_2 emissions, but T4 did not differ from T1. BC reduced N_2O emissions from sandy and clayey soils relative to T1. BC with FC and V affected pH, CEC, P and base contents. However, those effects were higher in sandy soil. The BC suppressed N_2O from V and FC. In Chapter 4, BC decreased N_2O from N fertilization compared to only N fertilizer. T4 had higher tillering and grain yield. Also, T2 to T4 had higher 100-grain weight and shoot. T3 and T4 had the highest N in grains. The application of BC to soil improves N availability and use efficiency, enhances grain yields and reduces N_2O from N fertilization. This study opened encouraging perspectives to the evaluation of sugarcane straw BC to improve soil quality and mitigate GHG emissions.

Keywords: Carbon dioxide; Carbon sequestration; Climate change; Methane; Nitrous oxide; Pyrogenic carbon; Soil management; ^{15}N -tracer method

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1 INTRODUCTION

An increasing number of global issues such as climate change, poverty, declining agricultural production, scarcity of water, fertilizer shortage and food security have been prominent in political and policy debates. The need to tackle these issues creates an increasing demand for solutions to be implemented now or in the near future in order to produce effects on a global scale. This is an urgent task that cannot be accomplished by any single technology, but requires many different approaches (CERNANSKY, 2015).

Sustainable management of the soil environment is vital to maintain its functions (i.e. biomass production, biodiversity pool, regulation of major elemental cycles, storing and filtering of substances and water, carbon pool etc) as an entity that sustains plants, animals and humans (SEYBOLD; HERRICK; BREJDA, 1999; MCBRATNEY; FIELD; KOCH, 2014). Soil organic carbon (SOC) plays a crucial role in many of these soil functions and it has been considered a “universal indicator” of soil security (STOCKMANN et al., 2013), since organic matter (OM) inputs provide benefits to soil functions by improving soil chemical (SANT’ANNA et al., 2009), physical (PAPADOPOULOS et al., 2014) and biological properties (SANTOS et al., 2012).

One such approach is the mitigation of greenhouse gases (GHG) emissions from agriculture. Ever since, agriculture and climate change are characterized by a complex relationship of cause and effect. Agriculture is influenced by climate change with negative impacts on crop yield and increased costs of cultivation (BRILLI et al., 2014). On the other hand, the intensification of agriculture can be a major contributor to global warming through activities that enhance the emission of GHGs (IPCC, 2014).

The main GHGs after water vapor are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). The current atmospheric concentrations of CO_2 , CH_4 , and N_2O are 40%, 150%, and 20% higher than those during the pre-industrial era (1750), reaching 392 *ppmv* (vs. 280 *ppmv*), 1,750 *ppbv* (vs. 700 *ppbv*), and 324 *ppbv* (vs. 270 *ppbv*), respectively (IPCC, 2014). During the 20th century, global surface temperature had a increase of approximately 0.6°C to 0.7°C and is projected to likely exceed 1.5°C by the end of the 21th century (2081–2100) (IPCC, 2014). N_2O and CH_4 emissions are of great importance due to its high global warming potential: 298 and 25 times greater than CO_2 , respectively.

Globally, agricultural lands account for about 25% of the CO_2 , 50% of the CH_4 , and 70% of the N_2O emissions (HUTCHINSON; CAMPBELL; DESJARDINS, 2007). Data from the Brazil’s second GHG inventory (BRASIL, 2010) showed that emissions of CH_4 and N_2O associated with the agricultural sector accounted, respectively, for 71% and 87% of the total GHG emissions in 2005. Similarly, the enhanced clearing and burning of forests for other uses, particularly agriculture, represents 77% of the total emissions of CO_2 (BRASIL, 2010). In the face of the dual challenge to mitigate global climate change and ensure food security for a growing population, management strategies that build soil organic matter (SOM) have received considerable attention (STOCKMANN et al., 2013).

The conversion from native forest to crops reduces the potential of soil to act as a sink in the carbon cycle (HARTEMINK, 2008; KASCHUCK; ALBERTON; HUNGRIA, 2011). Stockmann et al. (2013) estimated that to date, 30% to 75% of the original soil organic carbon (SOC) level has been lost from agricultural soils. This depletion on SOC is primarily due to the removal of the natural vegetation, lower quantity and quality of organic inputs and exposure of SOM to microbial activity (SILVA et al., 2007). As a threshold of cumulative emissions is likely to be reached beyond which no level of emissions can be considered safe, strategies that can actually absorb carbon dioxide from the atmosphere will become more essential than others (WOOLF et al., 2010). Because photosynthesis converts CO_2 to organic carbon (OC), increases in plant carbon (C) stocks reduce atmospheric CO_2 concentrations.

As a strategy for climate change mitigation, the conversion of readily decomposable plant biomass to a relatively non-degradable organic matter (OM) may enhance soil C storage and removes CO_2 from the atmosphere. The process of heating biomass (e.g., woods, leaves, manure, sludge etc.) in the absence of oxygen (O_2), called pyrolysis, and application of the resulting material to agricultural or forest soils is being advocate as a C storage strategy in the last decades (SOHI et al., 2010). The solid product of pyrolysis, called “biochar” (BC) in the context of improving soil properties as part of agronomic or environmental management, also got into focus as a climate mitigation strategy.

Biochar can slow down the decay of the captured carbon dioxide in plants that would otherwise be fully returned to the atmosphere (SCHOLZ et al., 2014). Stavi & Lal (2012) estimated that the global capacity for sequestering C in soil amended with BC may range from 7 to 110 Pg. The International Biochar Initiative (IBI) suggested the inclusion of BC in climate mitigation policies and recently requested the incorporation of BC in the UNFCCC agriculture mitigation program (GURWICK et al., 2013), thus being considered a promising alternative to mitigate GHG emissions (CAYUELA et al., 2013) and increase the long-term stabilization of SOC (ZIMMERMAN; GAO; AHN, 2011). Additionally, there is evidence that BC amendment causes fundamental changes in soil nutrient cycles, often resulting in increased crop production, particularly in acidic and highly weathered soils (PROMMER et al., 2014).

1.1 Pioneering works and state-of-knowledge in biochar research

Charcoal production is a popular and ancient industry in Asian countries. During earlier times, there were evidences based on observations and anecdotes of the effects of various kinds of charred organic residues, especially rice husk, on wild plants and crops. However, these residues were carbonized in traditional kilns to be used as a fuel. Ancient Japanese texts showed evidences on the benefits of charcoal use in agriculture. Miyazaki (1697 apud OGAWA; OKIMORI, 2010) described that “After charring all waste, concentrated excretions should be mixed with it and stocked for a while. When you apply this manure to the fields, it is efficient for yielding any crop”.

Hundreds of years later, researchers in Japan started to encourage new uses of charcoal Kishimoto & Sugiura (1980 apud LEHMANN; JOSEPH, 2009). Makoto Ogawa began studies on the utilization of charcoal in agriculture and reported the effects of bark charcoal on soybean cultivation and pine tree rehabilitation (OGAWA; OKIMORI, 2010). In Brazil, ancient Amerindian populations also used charcoal-making methods and produced soils with an anthropic A horizon, known as “*Terra Preta*”, on the Amazon Basin. The first descriptions of “*Terra Preta*” occurred in 1870, and at the end of the 19th century its formation had been associated to human activities. Between the 1940s and 1970s, researchers proposed theories regarding the origin of these soils and showed that most of the stabilized *C* came from the conversion of organic wastes into charcoal (GLASER et al., 2000; GLASER; LEHMANN; ZECH, 2002).

The archeological sites with “*Terra Preta*” served (intentionally or accidentally) as deposits of plant material (i.e. leaves, peels, seeds etc.) and animal residues (i.e. bones, blood, fat, excreta, shells etc.). This complex organic waste resulted in the formation of highly fertile soils with high *P* levels ($> 1000 \text{ mg kg}^{-1}$ soil), *Ca*, *Mg*, *Zn*, *Mn* and *C* (KERN, 1996), originating a favorable environment for cation exchange reactions, which is a highly desirable characteristic when producing soil amendments for tropical soils. Thus, Sombroek et al. (2003) started to promote the idea of reproducing the main aspects of “*Terra Preta*” in agricultural soils, which inspired the biochar concept (GLASER et al., 2001; LAIRD et al., 2009).

Research in BC has grown rapidly since 2009/2010, and the emergence of international initiatives dedicated to BC research, technology and policy reinforces the interest in using BC not only for environmental management but also for a whole range of products (SCHMIDT, 2012). Despite BC science is advancing, most of the available data indicate inconclusive and contradictory results on the impacts of BC as a soil amendment (MUKHERJEE; LAL, 2014). Thus, the use of BC in agriculture is growing worldwide without the basic understanding of when, how and why BC can be a promising tool to improve soil quality, enhance *C* sequestration, and increase agronomic yield (STEINER et al., 2008).

1.2 Aim of this thesis and research questions

The major purpose of the researcher was to investigate the effects of BC application on soil chemical attributes, as well as understanding the mechanisms towards GHG emissions. In the work described in this thesis the researcher aimed to answer the following research questions:

- Does biochar produced from sugarcane straw present desirable characteristics as a soil amendment and potentially provide soil carbon sequestration?
- Does biochar produced from sugarcane straw combined with organic fertilizers have synergistic effects on soil chemical attributes and on the mitigation of greenhouse gases emissions?

- Does biochar produced from sugarcane straw mitigate nitrous oxide emissions and changes nitrogen availability to plants?

1.3 Thesis outline

The above research questions have been addressed in from chapters 2 to 4.

In chapter 2, it were assessed the basic chemical, physical and morphological properties of sugarcane straw biochar, as well as its priming potential on soil native carbon decomposition under laboratory conditions;

In chapter 3, it were investigated the interactions between sugarcane straw biochar and organic residues from sugar and bioethanol industry (filter cake and vinasse) in relation to improvement in soil chemical attributes, as well as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) emissions under laboratory conditions; and

In chapter 4, it was evaluated the potential of biochar on the mitigation of nitrous oxide (N_2O) emissions and availability of nitrogen to wheat plants in a greenhouse pot experiment.

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2 CHARACTERIZATION OF SUGARCANE STRAW BIOCHAR AND ITS EFFECT ON NATIVE SOIL ORGANIC MATTER DECOMPOSITION IN A SANDY SOIL MATRIX

Abstract

Biochar BC plays a major role in expanding options for sustainable soil management. However, its composition and stability in soil can vary among types of biomass, pyrolysis conditions and soil type. This study investigated the chemical and physical properties of sugarcane straw BC, as well as its priming potential on native SOC. The feedstock was collected in a sugarcane area within a mill located in Piracicaba, Brazil, and submitted to slow pyrolysis at 450°C . Both feedstock and BC were characterized in relation to their physical and chemical properties. Additionally, it was evaluated the carbon stability of the BC in a sandy soil matrix (Quartzipsamment) by evaluating CO_2 fluxes and $^{13}\text{C} - \text{CO}_2$ in a laboratory incubation with soil, BC and soil + BC mixtures with application rates equivalent to 10, 20 and 50 Mg ha^{-1} of BC; T1, T2 and T3, respectively. BC presented C and N contents 41% and 75% higher, respectively, compared to the feedstock. Total K, Mg and P increased by 30%, 110% and 126%, respectively. It was observed structural similarity between the biomass and BC, with preserved vessel elements, stomata, trichomes and chitin from fungal hyphae. The lowest CO_2 fluxes were observed for the BC, and the CO_2 fluxes from soil and soil + BC did not differ. The highest $\text{CO}_2 - \text{C}_4$ emission was observed in the first day of incubation, and there were no differences in the $\text{CO}_2 - \text{C}_3$ emissions among treatments. The sugarcane straw pyrolyzed at 450°C presents desirable characteristics to promote improvements in soil chemical attributes. The stability of the sugarcane straw BC in a sandy soil is an opportunity for carbon sequestration in soils with low capacity to stabilise carbon.

Keywords: Biomass; Priming effect; Pyrolysis; Soil organic matter; $^{13}\text{C} - \text{CO}_2$; ^{13}C natural abundance

2.1 Introduction

BC is a carbon-rich material produced from the thermal degradation of biomass under anoxic conditions, a process called pyrolysis. During the formation of BC through pyrolysis three pathways can occur: (i) Formation of gas and BC; (ii) Formation of tars and liquids; and (iii) Carbonization and Gasification. Thus, BC is the result of solid-phase reactions, in which devolatilized biomass leaves behind a carbonaceous residue (primary BC), and decomposition of organic vapours (tars) to form coke (secondary BC). The occurrence and partition between these stages depend on a few factors, such as: (i) final temperature; (ii) removal rates of volatile compounds; and (iii) residence time of pyrolysis process (SOHI et al., 2009).

During pyrolysis, after complete depolymerization of cellulose and hemicellulose occurs the formation of an amorphous C matrix, and with increasing temperature there is an increase in aromatic C. Above 600°C (carbonization and gasification) the remaining non-C atoms

volatilize and occurs the nucleation of graphene sheets (AMONETTE; JOSEPH, 2009). Additionally, a combination of pyrolysis conditions and occurring catalysts in biomass yield a wide variety of organic compounds (secondary reactions), which include aldehydes, ketones, carboxylic acids, alcohols and anhydrosugars from cellulose pyrolysis (MOHAN; PITTMAN JR; STEELE, 2006). Although *C* is the major constituent of BC, its exact properties as well as the quantity of each pyrolysis product, depend upon the quality of the feedstock and the pyrolysis conditions.

Plant materials are mostly composed by cellulose, hemicellulose and lignin, with smaller quantities of other organic (e.g. resins, fatty acids, phenolics and phytosterols) and inorganic (compounds with mineral *N, P, K, S, Cl, Si* etc.) compounds. These constituents can vary considerably among types of biomass and the resulting composition of the BC relies primarily on the chemical and thermal stability of these constituents (MANNING; LOPEZ-CAPEL, 2009). Pyrolysis conditions comprise heating rate, final temperature, pressure, residence time, pre-treatment (e.g. drying, chemical activation etc.), flow rate of ancillary inputs (e.g. CO_2 , O_2 , steam etc.), and post-treatment (e.g. crushing, sieving, activation etc.). Although all of these parameters contribute to the final BC structure, the final temperature of pyrolysis seems to be the most important regarding physical changes, due to the release of volatiles and the formation and volatilization of intermediate melts.

An additional factor that contributes to the complexity of BC is the influence of molecular structure on BC morphology. According to Schimdt & Noack (2000), black carbon (which includes BC) “represents a continuum from partly charred material to graphite and soot particles with no general agreement on clear-cut boundaries”. Consequently, this continuum of charring conditions leads to distinct degrees of oxidation and stability, which are often assessed through changes in the concentrations of *C, H, O* and *N* and their elemental ratios (KRULL et al., 2009).

Sollins, Homann & Caldwell (1996) described that the stability of the SOC is the result of three general sets of characteristics: (i) Recalcitrance, which comprises the molecular-level organization of organic substances, including elemental composition, presence of functional groups and molecular conformation; (ii) Inter-molecular interactions between organics and inorganic substances or other organic substances, which may alter the rate of degradation of those organics or synthesis of new organics; and (iii) Accessibility, which refers to the location of organic substances with respect to microbes and enzymes. For BC, recalcitrance is assumed to be the most important phenomenon for long-term of *C* sequestration.

The stability and resistance of BC against biotic and abiotic oxidation is highly variable, since experimental results reported both rapid (MAJOR et al., 2010) and slow (KUZUYAKOV; BOGOMOLOVA; GLASER, 2014) decomposition of biomass-derived BC in soils. Despite the fact that the decomposition pathways of black carbon (which includes biochar) in soil is still a matter of debate (SCHMIDT et al., 2011), there are evidences that these processes lead not only to mineralization of this highly stable form of organic *C* in soil, but also may create negatively-charged surfaces over time, consequently leading to changes in availability of plant nutrients and

cation exchange properties (GLASER; LEHMANN; ZECH, 2002; LIANG et al., 2006). Thus, quantifying the extent of BC decay will provide important information regarding the timeframe over which benefits from BC to soil application are delivered, besides the understanding of the processes influencing BC disappearance (LEHMANN et al., 2005).

A part of the mineral phase of the feedstock remains in the BC after pyrolysis and varies according to the ash content of the feedstock. In high-ash BC, one important aspect is the high value of pH (> 7) due to soluble salts (e.g. K and Na carbonates and oxides) derived from the feedstock biomass and sorbed in the BC structure during pyrolysis, thereby influencing in soil nutrient status and liming capacity. Kloss et al. (2012) found the highest salt (4.92 mS cm^{-1}) and ash contents in straw-derived BCs.

Other alleged benefits on soil properties through BC addition may occur by: (i) sorption of heavy metals and organic substances (FREDDO; CAI; REID, 2012); (ii) increase in soil water retention (SAARNIO; HEIMONEN; KETTUNEN, 2013); (iii) favorable medium for microbial activity (JAAFAR; CLODE; ABBOTT, 2014); (iv) rehabilitating degraded lands and bringing marginal soils into production (BARROW, 2012); (v) reduction of nutrients losses by leaching (ANGST et al., 2013); (vi) increase potential of soil C sequestration (STAVI; LAL, 2012); and (vii) mitigation of N_2O and CH_4 emissions to the atmosphere (CAYUELA et al., 2013; SÁNCHEZ-GARCÍA et al., 2014; PROMMER et al., 2014).

Given the above considerations, the adoption of BC-based strategies may occur in multiple sectors to varying extents because BC systems serve to address different objectives on different scales. In a broad sense, there are four complementary and often synergistic objectives that may motivate BC application for environmental management: soil improvement; residue management; climate change mitigation; and energy production (LEHMANN; JOSEPH, 2009).

Managing animal and crop residues from agriculture is considered as environmental and economic opportunity, since these residues and other by-products are usable resources for pyrolysis bioenergy (BRIDGWATER, 1999, 2003). The annual production of crop residues in the world is estimated in 3.8 Pg of dry matter (LAL, 2005), 75% from cereals and 8% from sugarcane. Currently, the production of sugarcane generates about 314 Tg yr^{-1} of residues (WOOLF et al., 2010), and Brazil ranks as the largest producer of the crop, with production of crop residues estimated in 175 million tons (CONAB, 2013). In addition, the intensification of green cane instead of burning has led to greater deposition of post-harvest residues (i.e. leaves and tips) on soil surface, ranging between 10 and 20 Mg ha^{-1} of dry matter.

The growing demand to furnish feedstock for biofuel emphasizes the importance of carefully evaluating ecological trade-offs. One is appropriate management of organic residues and byproducts of bioenergy (e.g. BC), which can improve soil quality and productivity (GAUNT; LEHMANN, 2008) besides acting as a climate mitigation strategy (GURWICK et al., 2013). In this context, BC is able to play a major role in expanding options for sustainable soil management and achieving net reductions in GHG emissions on a local and global scale.

The feedstock selected to produce the BC evaluated in this thesis was sugarcane straw, which was converted to BC through pyrolysis under controlled conditions. The description of the production and general characteristics of the feedstock is addressed in the next section.

2.2 Material and Methods

2.2.1 Collection of the feedstock

The sugarcane straw was collected in a sugarcane area within a mill located in Piracicaba, State of Sao Paulo, Brazil. A recently harvested area was selected and therefore presented a large amount of post-harvest residues on soil surface ($\approx 10 \text{ Mg ha}^{-1}$ of dry matter).

2.2.2 Pyrolysis conditions

Before pyrolysis, the straw particles were cut into fragments of 5 cm. Then, the reactor was cleaned under heating with air injection in order to remove impurities prior the allocation of the raw material. Approximately 3 kg of feedstock biomass were manually placed into the sample port. The pyrolysis process began with the activation of electrical resistors. The final temperature was 450°C with heating rate of $10^{\circ}\text{C min}^{-1}$. After that, the resistors were handled by automatic controllers, which held temperature relatively constant ($\Delta \approx 20^{\circ}\text{C}$) for a retention time of 2 hours to ensure complete carbonization of the feedstock.

After completion of the pyrolysis, the heaters were turned off and the reactor was allowed to cool before unloading the BC. The sample presented homogeneous carbonization, with a volume reduction of 30% to 40%. The pyrolysis process yielded 30% of BC, 40% of liquids (bio-oil) and 30% of gas, which is within the range observed in most studies (LAIRD et al., 2009).

2.2.3 Basic physical, chemical and morphological characterization

The characterization of the BC in the present study was performed according to the International Biochar Initiative (IBI) Standards version 1.1 (IBI, 2013), developed by an international committee that establishes a standardized product definition and guidelines for characterization and commercialization of BC applied to soils. The physical and chemical characterization of the BC were performed in the Department of Soil Science at ESALQ/USP.

Ash, volatile matter (*VM*) and moisture were determined according to the ASTM D3174-11 (ASTM, 2011) and D3175-11 protocols (ASTM, 2007b). The particle size distribution was done according to the ASTM D2862-10 protocol for activated carbon (ASTM, 2007a). The electrical conductivity (*EC*) was performed using a BC:solution ratio of 1:2 (w:v) and agitation at 220 rpm for 30 minutes. After resting for 16 hours, the samples were filtered and the *EC* was measured with a conductivity probe. The specific surface area (*SSA*) was determined as proposed by Cerato & Luttenegger (2002), which is a standard method of the SSSA for

estimating *SSA* of soils and clays, and consists in oven-drying the samples and subsequent saturating with ethyleneglycol monoethylether (*EGME*). The excess of the solvent is removed in desiccators connected to a vacuum pump until the *EGME* has formed a monomolecular layer on the surface of the material.

The water holding capacity (*WHC*) of BC was evaluated by weighing 25 g of dry material on a filter paper packed in glass funnel. Then, in each funnel was added 250 ml of deionized water gradually, and during 24h was observed the drainage of the water. The *WHC* was calculated as follows: $WHC = ((250 - \text{volume of drained water})/25g \text{ of dry residue})$, in $L\ kg^{-1}$. Additionally, we determined *pH* in $CaCl_2$ 0.01 *M* and apparent density (EMBRAPA, 1997). The multielemental analysis was performed primarily with the acid digestion of the material (“modified dry-ashing”), as proposed by Enders & Lehmann (2012). Then the extracts were submitted to multielemental analysis using ICP-OES. Results were converted to a dry-mass basis according to the following equation:

$$\omega E = \frac{V}{M} \times \rho E \times F, \quad (1)$$

where:

ωE is Proportion by mass of the element in the solid matter ($\mu g\ g^{-1}$);

M is Mass of the sample used (mg);

V is Volume of the extract (mL);

ρE is Concentration of the element ($\mu g\ L^{-1}$); and

F is Conversion factor ($1\ L\ mL^{-1} \times mg\ g^{-1}$).

After grounding and sieving the BC particles to 100 mesh (0.149 *mm*), the concentrations of *C*, *H* and *N* were determined by dry combustion. The oxygen content (*O*) was determined as follows: $O(\%) = 100 - (C - N - H - ash)$. The isotopic abundance of ^{13}C and ^{15}N of soil and BC, as well as for gas CO_2 , were determined as relative difference in isotope ratios, $R(^{13}C/^{12}C)$ and $R(^{15}N/^{14}N)$ respectively, between samples and those of the International Measurement Standards, and expressed as delta values (δ).

The two former were measured by using an elemental analyzer (Carlo Erba CHN-1110, Milan, Italy) coupled to a Isotope Ratio Mass Spectrometer (IRMS) (Thermo Scientific Delta Plus, Bremen, Germany), while the later was measured using a trace gas pre-concentrator (Thermo Scientific PreCon, Bremen, Germany) coupled to the IRMS, in the Laboratory of Isotope Ecology at CENA/USP. The isotope $R(^{13}C/^{12}C)$ and $R(^{15}N/^{14}N)$ ratios of the BC were calculated as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = \frac{R_{spl} - R_{std}}{R_{std}}, \quad (2)$$

where:

R_{spl} is isotope-number ratio of carbon $R(^{13}C/^{12}C)$ and nitrogen $R(^{15}N/^{14}N)$ of the sample; and

R_{std} is isotope-number ratio of carbon $R(^{13}\text{C}/^{12}\text{C})$ of the International Reference Material VPDB , $R(^{13}\text{C}_{\text{VPDB}}) = 0.0111802$, or, of nitrogen $R(^{15}\text{N}/^{14}\text{N})$ of the ambient air, $R(^{15}\text{N}_{\text{AIR}}) = 0.0036765$.

The morphology of the BC produced was investigated by scanning electron microscopy (*SEM*) in the Laboratory of Electron Microscopy and Microanalysis at ESALQ/USP, using the instrument LEO 435 VP. Before examinations, the BC particles were fixed on stubs with graphite liquid and sputter-coated with gold to ensure the most effective electrical conduction and produce high-quality images, using the equipment Sputter SCD 050 BALTEC.

2.2.4 Stability of sugarcane straw biochar in a sandy soil matrix

A laboratory incubation was designed to assess the stability of the sugarcane straw BC in a sandy soil matrix, by measuring CO_2 emission and $\delta^{13}\text{C} - \text{CO}_2$ in soil + BC mixtures ($C3 - C4$). The soil used was classified a Quartzipsamment collected from a native vegetation area in the 0-20 cm layer. Before incubation, the soil was air-dried, homogenised, and sieved to 2 mm. The treatments applied to the incubation units and basic chemical characteristics of the incubation soil are described in Tables 2.1 and 2.2, respectively.

The application rates of BC used in the soil + BC mixtures were based on Woolf et al. (2010), in which the maximum field recommended dose is 50 Mg ha^{-1} . Before installing the experiment, a small incubation containing only the soil + 50 Mg ha^{-1} treatment ($T3$) was tested in order to verify the occurrence of kinetic isotope effect.

The incubation units with soil + BC consisted of 100 g soil and 0.38 g ($T1$), 0.77 g ($T2$) and 1.92 g ($T3$) of BC. These additions represent a field application rate of $10 - 50 \text{ Mg ha}^{-1}$ (assuming an incorporation depth of 20 cm and a bulk density of 1.3 g cm^{-3}). These were then pre-incubated in a refrigerator overnight and placed in airtight glass jars of 0.55 liter at 25°C .

Table 2.1 – Description of the evaluated treatments in the laboratory incubation with soil, BC, and soil + BC mixtures

Treatments	Description
Soil	Soil-alone [†]
Biochar	BIOCHAR alone [‡]
$T1$	Soil + BIOCHAR 10 Mg ha^{-1} §
$T2$	Soil + BIOCHAR 20 Mg ha^{-1} §
$T3$	Soil + BIOCHAR 50 Mg ha^{-1} §

[†] 100 g of soil for each incubation unit (except $T2$).

[‡] 1.28 g of BC, mass equivalent to the C content of the soil used in the experiment (See Table 2.2).

§ 0.38, 0.77 and 1.92 g of BC, respectively.

The jars were flushed with synthetic air immediately after closure and refilled in each sampling event to minimize the CO_2 concentration and to maintain the atmospheric pressure

within the jars. Gas samples were collected using 20 ml syringes after 1, 7, 13, 25, 31 and 37 hours of accumulation, and the concentration of CO_2 was determined using a gas chromatograph. The results were used to estimate the fluxes of CO_2 ($mg\ CO_2 - C\ m^{-2}\ h^{-1}$) based on the following equation proposed by Clayperon (1834):

$$P \times V = n \times R \times T, \quad (3)$$

where:

P is atmospheric pressure (atm);

V is volume occupied by gas (headspace) (μmol or $nanomol$);

n is moles of the gas;

R is universal gas constant = $0.082\ (L\ atm\ K^{-1}\ mol^{-1})$; and

T is absolute temperature (K).

To determine the $\delta^{13}C - CO_2$, a portion of the gas samples was expanded in a vacuum line, in which subsamples were taken with a high precision glass syringe and analyzed via coupling a PreCon to an Isotope Ratio Mass Spectrometer. A two-source mixing model was used to estimate the partition of the CO_2 fluxes into its respective components (soil and BC), based on the differential ^{13}C discrimination by C3 (soil) and C4 (BC) sources. The following equation was used:

$$f_{soil} = \frac{R_{mixture} - R_{biochar}}{R_{soil} - R_{biochar}}, \quad (4)$$

where:

f_{soil} is proportion of CO_2 derived from the C3 source (soil) in a soil + BC mixture;

$R_{mixture}$ and R_{soil} is $R(^{13}C/^{12}C)$ of the CO_2 emitted from the soil + BC mixture and pure soil, respectively; and

$R_{biochar}$ is $R(^{13}C/^{12}C)$ of the CO_2 emitted from the C4 source (pure BC).

Table 2.2 – Total C and N , C/N ratio and $\delta^{13}C$ of the incubated soil used to test the stability of BC carbon

C	N	C/N	$\delta^{13}C$
%			‰
0.9 ± 0.1	0.1 ± 0.0	9.1 ± 0.1	-27 ± 0.1

Mean $\pm$ standard deviation ($n = 3$).

2.2.5 Statistical analyses

The t-test was performed for comparison between the mean values of biomass and BC results regarding their basic characterization and total concentration of nutrients. Analysis of variance (ANOVA) was applied to the results regarding the CO_2 fluxes and the Tukey's test

($P < 0.05$) was applied for the comparison of mean values between treatments. All statistical analyses were run using the software R (R Core Team, 2014).

2.3 Results and Discussion

Table 2.3 presents the results of physical, chemical and bromatological characterization of the feedstock (sugarcane straw) and the final product (BC). The cellulose and hemicellulose content decreased by 81% and 89%, respectively, while there was a relative increase in the lignin (5 times higher compared to the feedstock) (Table 2.3) ($P < 0.01$). Hemicellulose is the first to decompose during pyrolysis, beginning at 220°C and substantially completed by 315°C . Cellulose does not decompose until 315°C . Although lignin begins to decompose at 160°C , it is a slow and steady process extending to 900°C and yields a solid residue approaching 40% by weight of the original sample (MOHAN; PITTMAN JR; STEELE, 2006).

The BC presented a C content 41% higher compared to the feedstock, while the O and H contents decreased by 41% ($P < 0.01$) and 54%, respectively (Table 2.3). The increase in the C content with increasing pyrolysis temperature is often associated with H and O loss from BC (ANTAL; GRØNLI, 2003). Pyrolysis between 250°C and 600°C increases the proportion of C in the solid phase from about 40% to 50% by weight to on the order of 70% to 80% by weight, consisting of an intimate mixture of two solid C phases (i.e. amorphous C and graphene sheets). Higher temperatures increase the C content to more than 90% by weight, except for high mineral-ash BCs (ANTAL; GRØNLI, 2003), creating thermally fixed C structures (AMONETTE; JOSEPH, 2009). Thus, the high C content, which the major proportion is a non-labile form, potentially increases soil C sequestration, reducing the accumulation of CO_2 in the atmosphere and effectively acting as a soil C sink.

The lower H/C and O/C ratios observed in the BC compared to the feedstock (Table 2.3) seem to be indicative of stability of pyrolysis BC (KRULL et al., 2009; SPOKAS; BAKER; REICOSKY, 2010), as is often illustrated in van Krevelen diagrams (BALDOCK; SMERNIK, 2002). Crombie et al. (2013) showed that O/C molar ratio correlated well with the accelerated oxidation of BC with H_2O_2 . Increasing pyrolysis temperature ($> 350^{\circ}\text{C}$) produces BC with H/C and O/C molar ratios below 0.5 and 0.2, respectively. According to Spokas et al. (2012), O/C molar ratio lower than 0.2 appears to provide at minimum, a 1,000-year BC half-life; however the study highlighted the influence of factors other than pyrolysis temperature on BC residence in soil such as feedstock biomass and post-production conditioning/oxidation. Additionally, changes in elemental ratios are accompanied by changes in the C functional groups, and thus reflect the molecular composition of the BC (BALDOCK; SMERNIK, 2002).

The results showed that the pyrolysis temperature did not result in the loss of N , showing a 75% increase compared to the feedstock (Table 2.3) ($P < 0.05$), which is within the range of total N content of BCs reported in literature, from 0.09% to 3.3% (CHAN; XU, 2009).

Table 2.3 – Physical and chemical characteristics of the feedstock (sugarcane straw) and BC produced from sugarcane straw pyrolysed at 450°C

Properties	Biomass	BC	P-value
Cellulose [†] ($g\ kg^{-1}$)	274.2 ± 4.7	52.2 ± 6.0	< 0.0001**
Hemicellulose [†] ($g\ kg^{-1}$)	400.1 ± 7.3	45.67 ± 4.3	< 0.0001**
Lignin [†] ($g\ kg^{-1}$)	130.51 ± 0.6	618.31 ± 6.4	< 0.0001**
<i>pH</i> [‡]	5.8 ± 0.1	9.2 ± 0.1	< 0.0001**
Moisture (%)	11.1 ± 2.3	2.6 ± 1.4	0.0267*
Volatile Matter (%)	90.5 ± 0.1	46.2 ± 2.4	0.0016**
Ash (%)	8.4 ± 0.1	14.5 ± 0.9	0.0005**
<i>CE</i> ($\mu S\ cm^{-1}$)	1848.8 ± 7.3	716.5 ± 64.8	0.0009**
¹⁵ N(‰)	..	3.3 ± 0.1	..
¹³ C(‰)	..	-13.4 ± 0.1	..
<i>C</i> (%)	47.9 ± 1.2	69.1 ± 2.5	0.0011**
<i>N</i> (%)	0.8 ± 0.2	1.2 ± 0.3	0.0331*
<i>H</i> (%)	6.1 ± 0.2	2.8 ± 0.0	–
<i>O</i> (%)	50.5 ± 0.5	30.0 ± 2.1	0.0020**
<i>C/N</i>	60.4 ± 0.4	57.6 ± 1.4	0.0017**
<i>H/C</i>	0.1 ± 0.0	0.02 ± 0.0	–
<i>O/C</i>	1.05 ± 0.01	0.4 ± 0.0	0.0012**
Density ($g\ cm^{-3}$)	0.2 ± 0.0	0.5 ± 0.1	–
Particle size distribution (%)	% > 4.760 μm	30.9	..
	% 2.380 – 4.760 μm ;	32.8	..
	% 420 – 2.380 μm ;	31.6	..
	% < 420 μm	4.7	..
<i>WHC</i> ($L\ kg^{-1}$)	..	1.5 ± 0.1	..
<i>SSA</i> ($m^2\ g^{-1}$)	..	435.0 ± 23.2	..

Mean ± standard deviation (n=3).

[†]Soest (1963) and Soest & Wine (1967).

[‡]*pH* in $CaCl_2$ 0.01M.

.. not available.

– no comparison between means in t-test.

* $P < 0.05$ and ** $P < 0.01$.

A survey comprising 80 published peer-reviewed papers summarized that total *N* content reached a maximum between 300 to 399°C in a wide range of BCs and decreased at greater temperatures (IPPOLITO et al., 2015). Studying the effect of temperature and holding time on *C* and *N* composition and *pH* values of sewage-sludge BC, Bagreev, Bandosz & Locke (2001) observed the same effect between 400 and 600°C. This can be related to two processes: (i) a concentration effect due to loss of other elements by volatilization (IPPOLITO et al., 2015); and (ii) chemical changes in the carbon phase, such as an increase in the degree of aromatization and the gradual incorporation of amine functionalities in the carbon matrix as heteroatoms such as pyridine-like structures (BAGREEV; BANDOSZ; LOCKE, 2001).

However, the availability of the converted *N* is likely to be minimal over time periods relevant to plant growth (CHAN; XU, 2009). The *C/N* ratio of the BC produced is within the wide range found in literature, between 7 and 400 with a mean of 67 (CHAN; XU, 2009). It is important to highlight the existing controversy on this subject, since recent studies have shown

contrasting results regarding the relationship between C/N ratio and N mineralization/immobilization with BC application to soils (THIES; RILLIG, 2009). Bagreev, Bandosz & Locke (2001) observed that the suggested chemical changes in N forms described above are accompanied by an increase in pH of BC by 3.8 units as a result of dehydroxylation of inorganic species. Specifically, increasing pyrolysis temperature removes acidic functional groups from the BC surface (NOVAK et al., 2009; LI; QUINLIVAN; KNAPPE, 2001), as well as nutrients in mineral form, or salts (such as KOH , $NaOH$, $MgCO_3$, $CaCO_3$, organic metal salts) separate from the solid organic matrix, resulting in higher ash content and its high pH (KNICKER, 2007).

The alkaline nature of the BC produced ($pH > 7.0$) (Table 2.3) is an extremely important factor in relation to their application to soil, serving as a liming agent (CHAN et al., 2007; KLOSS et al., 2012) and increasing nutrient bioavailability for a number of soil types (JONES et al., 2012). Oxygen-containing organic functional groups, ash (metal oxides) and carbonate minerals are the main forms of alkalinity in BCs (YUAN; XU, 2011b). BC may also contain significant amounts of soluble base cations (SINGH et al., 2010). However, the extent of alkalinity varies depending on feedstock type and pyrolysis temperature. Yuan & Xu (2011a) observed that BC derived from legume species showed greater potential to increase soil pH than the BC produced from non-leguminous plants. More recently, fused-ring aromatic structures and aromatic $C - O$ groups have been shown to correlate positively with the pH of BC (LI et al., 2013).

Water released by dehydroxylation reactions can act as a pore former and activation agent creating very small (angstrom-scale) pores in the carbonaceous deposit. Bulk density (BD) is also an important physical feature of BCs, with possible effects on soil water infiltration, root growth and soil fauna due to its application (MAJOR et al., 2010). It was observed a 150% increase in the BD of the BC compared to the feedstock (Table 2.3), which is due to the loss of volatile and condensable compounds and concomitant relative increase of graphitic structures during pyrolysis (EMMERICH et al., 1987), although is still a low density material due to its high porosity (OBERLIN, 2002). The BD of BCs given in literature range from 0.09 to 0.5 g cm^{-3} (KARAOŞMANOĞLU; ISIGİĞÜR-ERGÜDENLER; SEVER, 2000; ÖZÇİNEM; KARAOŞMANOĞLU, 2004; BIRD et al., 2008; SPOKAS et al., 2009), values much lower than those of soils.

In relation to the particle size distribution, the results indicate uniform particles in relation to the first three size classifications and a smaller percentage of particles < 420 microns (Table 2.3). The BC particle size is also highly dependent on the pyrolysis conditions and nature of the feedstock (DOWNIE; CROSKY; MUNROE, 2009). The shrinkage/swelling stresses and friction during pyrolysis seem to be concomitant with the loss of volatile matter and make BC particles smaller than that of the feedstock depending on the final temperature, pressure and heating rate (CETIN et al., 2004). This is also true in the post-handling of the material as the BC is significantly more friable than the original biomass.

The produced sugarcane straw BC is able to hold 50% more than its own weight in water (Table 2.3). Although the *WHC* of pure BC samples are highly variable and in some cases holding more than ten times its own weight in water (KINNEY et al., 2012), the hydrologic behavior of soil + BC mixtures are more meaningful. In other words, changing soil hydrologic properties fundamentally involves changing the shape, volume, and connectivity between and within soil particles.

When BC has a different shape, size and/or internal porosity compared to the amended soil, it may change soil hydrology (MASIELLO et al., 2015). According to measurements made by Barnes et al. (2014), soil hydraulic conductivity decreased by 92% in sand and 67% in organic soil, but increased by 92% in clayey soils with BC application. Thus, the capacity to retain water in BC-amended soils is a function of both the soil and BC properties.

The high surface area of the produced BC (Table 2.3) leads to its potentially useful properties including contaminant control and soil nutrient retention and release (MUKHERJEE; ZIMMERMAN; HARRIS, 2011). Numerous studies have shown that surface area tends to increase with pyrolysis temperature (VERHEIJEN et al., 2010). This is most often associated with physical and chemical changes, such as the removal of *H* and *O* containing functional groups (ZHAO et al., 2013) and thermal decomposition of cellulose and lignin (CHEN et al., 2012).

The macroporous structure (pores of approximately 1mm diameter) of BC produced from lignocellulosic plant material inherits the architecture of the feedstock, and is potentially important to water retention and adsorption processes of soil (OGAWA; OKIMORI; TAKAHASHI, 2006). The type of pyrolysis (fast vs. slow) also has a great influence on the BC surface area, as fast pyrolysis is likely to promote incomplete physico-chemical transformations, yielding a lower surface area BC compared to slow pyrolysis (IPPOLITO et al., 2015). Additionally, observations on fresh and aged BC concluded that the pore and surface structures of BC change over time and potentially provide suitable habitats for soil microorganisms (HOCKADAY et al., 2007; LEHMANN et al., 2011).

The primary evidences of chemical changes in the mineral phase of the BC during pyrolysis are the significant increase in ash content with concomitant steep decrease of the *EC* compared to the feedstock (Table 2.3). The higher ash indicates that much of the minerals in the feedstock are carried over into the BC structure and concentrated during pyrolysis (AMONETTE; JOSEPH, 2009), while the lower *EC* of the BC suggests changes in the availability of these nutrients in solution. Table 2.4 shows the total contents of macro and micronutrients of the feedstock (sugarcane straw) and BC produced from sugarcane straw pyrolysed at 450°C. Given the aforementioned, it is important to keep in mind that the total nutrients concentration cannot accurately predict their availability, since only a fraction of the total content may be readily available forms for uptake by plants.

There was an increase of 30%, 110% and 126% in *K*, *Mg* and *P* contents ($P < 0.05$), respectively, compared to the feedstock, while 67% of the total *Ca* was conserved in the BC ($P < 0.05$) (Table 2.4).

Table 2.4 – Total contents of *Ca*, *Mg*, *K*, *Na*, *P*, *S*, *Fe*, *Mn* and *Zn* of the feedstock (sugarcane straw) and BC produced from sugarcane straw pyrolysed at 450°C

Element	Biomass	BC	P value
		Concentration ($mg\ kg^{-1}$)	
<i>Ca</i>	7,670.9 ± 235.1	5,124.3 ± 926.8	0.0347*
<i>Mg</i>	1,536.5 ± 524.3	3,234.8 ± 683.6	0.0298*
<i>K</i>	9,521 ± 3,529.8	12,458.6 ± 2,523.3	0.3123
<i>Na</i>	4,143.2 ± 248.2	366.2 ± 43.1	0.0009**
<i>P</i>	982 ± 152.4	2,224.3 ± 449.7	0.0305*
<i>S</i>	431.7 ± 35.8	2,072.6 ± 451.5	0.0237*
<i>Fe</i>	2,995.1 ± 343.4	3,148.4 ± 314.4	0.5991
<i>Mn</i>	7,329.3 ± 1,232.7	146.7 ± 24.9	0.0096**
<i>Zn</i>	2,350.0 ± 266.3	32.1 ± 8.9	0.0043**

Mean ± standard deviation ($n = 3$).

* $P < 0.05$ and ** $P < 0.01$.

Gaskin et al. (2008) showed that the range of total *P*, *K*, *Ca* and *Mg* conserved varied from 60% to 100%, with bioavailability ranging from about 10% to upwards of 80% depending on the original biomass. Ippolito et al. (2015) showed that 55% and 65% of the *K*, *Mg* and *Ca* available from BCs can be related to total concentration.

Generally, slow pyrolysis (i.e. pyrolysis with slow heating rate) tends to produce BCs with greater *S*, available *P*, *Ca* and *Mg* compared to fast pyrolysis (BREWER et al., 2011). Additionally, increasing pyrolysis temperatures typically lead to a loss of easily decomposable substances, volatile compounds and elements (e.g., *O*, *H*, *N*, *S*) and thus concentrate nutrients present in BC, including *Ca*, *Mg* and *K* (KINNEY et al., 2012), thereby increasing ash content.

Although *K* is usually lost at relatively low temperatures, high contents of this element can be found in the BC due to its incorporation into the silicate structure, becoming less susceptible to volatilization Shinogi (2004 apud JOSEPH et al., 2013). Additionally, it is important to highlight the contribution of the feedstock for the BC's *K* content, since *K* is the nutrient extracted in the highest amounts by sugarcane plants (CANTARELLA; ROSSETTO, 2008). Cantrell et al. (2012) showed that total *K*, in combination with *Na*, was an important predictor of BC electrical conductivity, or the amount of salts present, indicating water solubility of the *K* in BC. In a comparison between 80 peer-reviewed articles, the availability of the total *K* in BCs ranged from 3.5 to 100% (IPPOLITO et al., 2015).

Calcium and Magnesium bond to complex organic molecules and can be lost by volatilization only in high temperatures (BOURKE et al., 2007). Only few information exist on the transformation of *P* during pyrolysis; however, some studies have shown an increase in

P concentration during pyrolysis. Knicker (2007) showed that *P* containing compounds volatilize near 760°C . Thus, at lower temperatures *P* availability is likely controlled by the coordinated cations present (Al^{3+} , Fe^{3+} , Ca^{2+} , Mg^{2+}), depending on feedstock (WAN et al., 2012). Due to its elevated *pH*, *P* in the BC will likely be associated with *Ca* and *Mg*, with some of these compounds in the readily available form. Data obtained from cited 2012 published data shows that available *P* ranges from 0.4% to 34% of total *P* in BC (IPPOLITO et al., 2015).

Iron (*Fe*) was strongly retained during the formation of BC ($P > 0.05$) (Table 2.4), which may have resulted from the reaction between the original biomass and the bed reactor during pyrolysis, or the presence of *Fe*-containing organic and inorganic compounds in the original biomass (AMONETTE; JOSEPH, 2009). In contrast, the total concentrations observed for *Mn* and *Zn* were lower than the feedstock ($P < 0.01$) (Table 2.4). Little information is available on the contents of trace elements in BCs; however, high concentrations of heavy metals have only been reported in BC produced from certain residues, such as sewage sludge and tannery wastes (BRIDLE; PRITCHARD, 2004), which are well known by the high amounts of these elements.

In relation to the morphological characterization of the BC, it is possible to observe that the final product (BC) presents structures of the original biomass. In the BC surface, the leaf structure of the sugarcane was maintained after pyrolysis, showing preserved stomata, trichomes and chitin from fungal hyphae (Figures 2.1 and 2.2). The porous structure of the BC imaged can be seen in Figure 2.3, in which it is possible to observe vessel elements (pores), punctuations and little obstruction of pores.

Figure 2.1 – Scanning electron micrograph of the biochar surface

As anticipated from the regular size and arrangement of plant cells in most biomass from which BCs are derived, the macropore size distribution is composed of discrete groups of pores sizes rather than a continuum (WILDMAN; DERBYSHIRE, 1991). To put this in perspective with typical soil particles, these discrete groups of pore diameters observed in this sample of $\approx 5\mu m$ to $10\mu m$, and $\approx 100\mu m$ compare to very fine sand or silt particle sizes, and fine sand particle sizes, respectively.

Figure 2.2 – Scanning electron micrograph of the biochar surface. (A,B) preserved estomata and trichomes, respectively, and (C) Presence of chitin from fungal hyphae

Additionally, it is important to highlight that these structures can serve as a favorable habitat for soil microorganisms. The cavities can be used as a refugee against predators and the presence of condensable compounds and labile *C* forms within these structures are important sources of *C* and energy for soil biota (LEHMANN et al., 2011). Microbial cells typically range in size from $0.5\mu m$ to $5\mu m$, and consist predominantly of bacteria, fungi, actinomycetes and lichens. Algae are $2\mu m$ to $20\mu m$ (BRADY; WEIL, 2002). The macropores present in the BC produced (Figure 2.3) may therefore provide suitable dimensions for clusters of microorganisms to inhabit.

The Figures 2.4 and 2.5 show the average daily and cumulative CO_2 fluxes measured along the incubation of soil-alone (Soil), BC-alone (BC) and soil + BC mixtures (*T1*, *T2* and *T3*), with the aim to study the stability of the BC in a sandy soil matrix. The BC treatment showed the lowest daily and cumulative emissions of CO_2 , and only a small fraction (less than 1%) of the applied biochar-*C* was mineralized (Figures 2.4 and 2.5). The daily and cumulative CO_2 emissions from Soil, *T1*, *T2* and *T3* were not different ($P > 0.05$) during the incubation period

Figure 2.3 – Scanning electron micrograph of a cross section of the biochar

(Figures 2.4 and 2.5, showing that the application rates of sugarcane straw BC used in this study did not result in increased decomposition or mineralization of native SOM relative to the non-BC treatment in a sandy soil matrix.

The effect of native SOM on biochar-*C* mineralization or the vice versa, called “priming effect”, is being recognized as an interactive process, i.e., each *C* source, BC and SOM affects the mineralization of the other (KEITH; SINGH; SINGH, 2011; ZIMMERMAN; GAO; AHN, 2011). Thus, studies have led to widely ranging results given the diversity of systems which have been studied, such as the proportion of labile *C* in BCs (LUO et al., 2010), amount and type of labile OM in soil (KEITH; SINGH; SINGH, 2011), the extent of BC ageing in soil (LIANG et al., 2010; CROSS; SOHI, 2011; ZIMMERMAN; GAO; AHN, 2011) and soil type among other factors.

Mineralization with BC application was found during short laboratory incubation periods and early portions of long-term studies (ZIMMERMAN; GAO; AHN, 2011; SINGH; COWIE, 2014). However, this effect diminished with time. Still other studies observed decreases in SOC mineralization with BC additions (KUZYAKOV et al., 2009; KEITH; SINGH; SINGH, 2011; JONES et al., 2012; STEWART et al., 2012; PRAYOGO; JONES; BAEYENS, 2013), and there are some studies that found that BC additions decreased SOC mineralization (CROSS; SOHI, 2011; WHITMAN; ENDERS; LEHMANN, 2014). Finally, many studies found no significant effect of BC additions on native SOC mineralization (KUZYAKOV et al., 2009; MUKOME; SIX; PARIKH, 2013; PRAYOGO; JONES; BAEYENS, 2013).

Figure 2.4 – Mean daily CO_2 fluxes measured over the incubation period (error bars are $\pm$ SD, $n = 3$). The treatments are represented as: (T1) soil + BC 10 Mg ha^{-1} ; (T2) soil + BC 20 Mg ha^{-1} ; (T3) soil + BC 50 Mg ha^{-1} . The control treatments (soil-alone and BC-alone) are presented as “Soil” and “BC”, respectively

The Figure 2.6 shows the partition of the CO_2 evolved from the soil + BC mixtures for each evaluation period, based on the $\delta^{13}C$ of the CO_2 emitted. The highest $CO_2 - C_4$ emission (BC) was observed in the first day of incubation in the highest BC application rate to soil (T3) ($P < 0.05$).

Figure 2.5 – Mean cumulative CO_2 fluxes measured over the incubation period (error bars are $\pm$ SD, $n = 3$). The treatments are represented as: (T1) soil + BC 10 Mg ha^{-1} ; (T2) soil + BC 20 Mg ha^{-1} ; (T3) soil + BC 50 Mg ha^{-1} . The control treatments (soil-alone and BC-alone) are presented as “Soil” and “BC”, respectively

The ^{13}C signature of the CO_2 evolved from $T3$ in the first day of incubation confirms that the BC contributed to 56% of the daily CO_2 flux in this treatment, contrasting from $T2$ and $T3$, whose CO_2 emissions from the BC were significantly lower, contributing for 27% and 36% of the daily CO_2 flux ($P > 0.05$), respectively. However, this effect diminished over time, with no differences between the $\text{CO}_2 - \text{C4}$ from $T1$, $T2$ and $T3$ in the subsequent evaluations ($P > 0.05$). Despite the higher $\text{CO}_2 - \text{C4}$ emission observed in the $T3$, there were no differences between the $\text{CO}_2 - \text{C3}$ (soil) emissions from $T1$, $T2$ and $T3$ in all evaluation periods.

This initial spike in CO_2 emission from BC may derive from a higher proportion of labile C compounds in the highest BC application rate (50 Mg ha^{-1}), as the 10 and 20 Mg ha^{-1} of BC did not present increased CO_2 fluxes. The same effect has been found in recent studies (SMITH; COLLINS; BAILEY, 2010; JONES et al., 2011; ZIMMERMAN; GAO; AHN, 2011).

Studying the effect of young BC on soil respiration, Smith, Collins & Bailey (2010) observed a short-term mineralization of biochar- C , which was attributed to the absorption of condensates (i.e. bio-oils) formed during pyrolysis when cooling the BC inside the reactor. These are likely the source of the labile C pool and thus not originated from the stable carbonized components of the BC (STEINER et al., 2007; NELISSEN et al., 2012; CLOUGH et al., 2013). Also, the C mineralized from BC may be a short-lived effect of moisture within the incubation units, once the penetration of water within BC pores could have induced a net release of trapped volatile compounds out of the pores.

Figure 2.6 – Partitioning of CO_2 evolution in soil + BC mixtures for each evaluation period (1, 7, 33, 54 and 90 days, error bars are $\pm \text{SD}$, $n = 3$). “C4” and “C3” signify the CO_2 evolved from BC and soil, respectively. The treatments are represented as: ($T1$) soil + BC 10 Mg ha^{-1} ; ($T2$) soil + BC 20 Mg ha^{-1} ; ($T3$) soil + BC 50 Mg ha^{-1}

Finally, recent studies have shown that increased CO_2 evolution, which is usually indicative of increased microbial activity, upon BC additions was actually partly due to

emission of inorganic C (i.e. carbonates) from within the applied BC (JONES et al., 2011). However, the C mineralization of the sugarcane straw BC was stimulated only in the first day of incubation and the turnover of soil native C due to BC application was repressed during all incubation stages, which means that the initial CO_2 release does not compromise the potential of sugarcane straw BC to sequester C in soil.

2.4 Conclusions

1. The sugarcane straw biochar pyrolyzed at $450^\circ C$ presents desirable characteristics to promote improvements in soil chemical attributes, such as its high pH , base contents and specific surface area; and
2. The isotope approach confirmed the carbon stability of the sugarcane straw biochar in a sandy soil matrix, which can be considered as an opportunity for carbon sequestration in soils with low capacity to stabilise carbon.

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3 EFFECTS OF BIOCHAR COMBINED WITH ORGANIC RESIDUES FROM SUGARCANE INDUSTRY ON SOIL CHEMICAL ATTRIBUTES AND EMISSIONS OF GREENHOUSE GASES IN TWO CONTRASTING SOILS

Abstract

The sugar and bioethanol industry generate large amounts of filter cake and vinasse, residues that are applied to sugarcane fields as conditioners and organic fertilizers. However, these residues may be significant sources of N_2O and CH_4 emissions to the atmosphere. This study assessed the impact of biochar (BC) on the emissions of CO_2 , CH_4 and N_2O promoted by filter cake and vinasse application, and investigated the synergistic effects of BC combined with these residues on the chemical attributes of two contrasting soils (i.e. clayey (Typic Hapludox) and sandy (Quartzpisamment) tropical soils). Two laboratory incubations were conducted for 100 days with the following treatments: (T1) soil + filter cake + vinasse; (T2) soil + filter cake + vinasse + BC 10 $Mg\ ha^{-1}$; (T3) soil + filter cake + vinasse + BC 20 $Mg\ ha^{-1}$; (T4) soil + filter cake + vinasse + BC 50 $Mg\ ha^{-1}$; and a control (soil-alone). Soil pH, P available and base contents increased and Al^{3+} decreased with BC addition to sandy soil, with no significant effects to clayey soil. Impacts of BC on the cation exchange capacity (CEC) were also more meaningful in sandy soil. Mineral N decreased with BC addition to both soils. Cumulative CO_2 emissions in T1 were 447% and 140% higher in sandy and clayey soils, respectively, than the control. Low and intermediate BC doses to sandy soil increased CO_2 emissions, but T4 did not differ from T1. Cumulative N_2O emissions in T1 were 537% and 125% higher in sandy and clayey soils, respectively. BC addition reduced N_2O emissions from 24% (T2) to 34% (T4) in sandy soil, and from 14% (T2) to 56% (T4) in clayey soil. BC combined with filter cake and vinasse presented synergistic effects on soil acidity, cation exchange properties, available P and exchangeable bases contents. However, the effects on soil amelioration were more evident in sandy soil than clayey, suggesting soil-specific BC interactions and its use not only as a conditioner but also as a fertilizer in nutrient-poor tropical soils. The BC suppressed N_2O emissions in contrasting soils treated with vinasse and filter cake, as a function of its application rate. Therefore, this material could be a potential option for maintaining and/or improving soil chemical properties while mitigating N_2O emissions to the atmosphere.

Keywords: Pyrogenic carbon; Carbon dioxide; Filter cake; Methane; Nitrous Oxide; Soil fertility; Vinasse

3.1 Introduction

Intensive agricultural and agro-industrial activities generate enormous amounts of organic residues. Recycling these materials in agriculture reduces the consumption of natural resources, while maintaining and/or improving the levels of nutrients and soil organic matter (WESTERMAN; BICUDO, 2005). The world annual production of crop residues is estimated in 3.8 Pg of dry matter (LAL, 2005), 75% from cereals and 8% from sugarcane.

In Brazil, the amount of crop residues generated from sugarcane cultivation is estimated in 175 million tons (CONAB, 2013), and the intensification of green cane harvesting has led to a greater deposition of leaves and tips on soil surface, ranging between 10 and 20 $Mg\ ha^{-1}$ of dry matter. Against the claiming demand to use this biomass for bioenergy generation, the Brazilian sugarcane sector have considered the partial removal of the post-harvest residues from soil surface without harming sustainability and yields (FRANCO et al., 2013).

In addition, the sugar and bioethanol industry generate other residues, such as filter cake and vinasse, which are fully recycled and largely applied to sugarcane fields as conditioners and organic fertilizers (MUTTON; ROSSETTO; MUTTON, 2008; PRADO; CAIONE; CAMPOS, 2013). Vinasse is an acidic ($pH \approx 4.5$) liquid waste of the ethanol distillation by fermentation, produced in an average of 13 L of vinasse per litre of ethanol, largely composed by water (95%), high amounts of organic carbon (OC) (6,000 - 23,000 $mg\ L^{-1}$) and mineral elements, mainly potassium (K). Filter cake is a residue from the filtration of sugarcane juice, produced in an average of 8 kg per ton of processed sugarcane (MACEDO; SEABRA; SILVA, 2008), contains 1% to 3% phosphorus (P) (CANTARELLA; ROSSETTO, 2008) and total C ranging from 420 $kg\ t^{-1}$ (RASUL et al., 2008) to 466 $kg\ t^{-1}$ (BUSATO et al., 2010).

Despite the benefits of filter cake and vinasse to conservation agriculture (MUTTON; ROSSETTO; MUTTON, 2008; PRADO; CAIONE; CAMPOS, 2013), recent studies have shown that these residues may be significant sources of greenhouse gases (GHG) emission to the atmosphere, mainly nitrous oxide (N_2O) and methane (CH_4) (CARMO et al., 2013; OLIVEIRA et al., 2013; PAREDES et al., 2013; SIGNOR; CERRI, 2013).

Nitrous oxide is a potent greenhouse gas (ca. 300 times that of CO_2) and the Brazilian Communication to the UN Framework Convention on Climate Change (UNFCCC) reported that 32% of the total N_2O emissions derive from the agricultural sector (BRASIL, 2010). Global emissions of N_2O increased from 10 to 12 $Tg\ N_2O - N\ yr^{-1}$ between 1900 and 2000 and may reach 16 $Tg\ N_2O - N\ yr^{-1}$ in 2050 (BOUWMAN et al., 2013), with 75% coming from gaseous loss of soil N through nitrification and denitrification processes (THOMSON et al., 2012). Methane has a radiative forcing approximately 25 times that of CO_2 (FORSTER et al., 2007) and its concentration has more than doubled in the atmosphere since pre-industrial times to a concentration of 1,750 $ppbv$ (IPCC, 2014).

Recently, BC has been studied as a potential agent to mitigate GHG emissions from agriculture. BC is a C-rich material, obtained from the thermal decomposition of biomass (e.g. wood, manure, crop residues) in the absence of O_2 , a process called pyrolysis. BC porosity, specific surface area, acid neutralizing capacity and redox properties vary with the feedstock and pyrolysis conditions and may alter the production of GHG in soil by biotic and abiotic mechanisms (SINGH et al., 2010; ENDERS et al., 2012).

Additionally, BC application to soil has shown positive effects on nutrient retention (GLASER; LEHMANN; ZECH, 2002; BÉLANGER et al., 2004; MAJOR et al., 2010), cation exchange capacity (CEC) (TRYON, 1948; MIKAN; ABRAMS, 1995; GLASER;

LEHMANN; ZECH, 2002) water retention (GLASER; LEHMANN; ZECH, 2002), composition and activity of soil biota (LEHMANN et al., 2011; CLOUGH et al., 2013), soil acidity (CHAN et al., 2008; GLASER; LEHMANN; ZECH, 2002), and electric conductivity when applied to soils (ASAI et al., 2009); all of which improves soil fertility (RONDON et al., 2007; NOVOTNY et al., 2009).

Despite the benefits associated to the agricultural use of BC, studies regarding the combination of this material with other organic residues are still limited. Schulz & Glaser (2011) found significant synergistic effects on plant growth when BC was combined with inorganic and organic fertilizers over the sole BC or fertilizer amendments.

According to Dias et al. (2010), the combination of BC with poultry manure reduced losses of *N* and produced high quality composts. Under these conditions, the combination of BC with organic fertilizers (or composts) may be an approach to improve nutrient cycling and reduce fertilizer application rates due to its effects on nutrient leaching and N_2O fluxes, and may fulfill non-agronomic purposes, such as reduction of GHG emissions (BLACKWELL; RIETHMULLER; COLLINS, 2009).

The aim of this study was to assess the effects of applying BC combined with residues from sugarcane industry (i.e. vinasse and filter cake) to soil chemical attributes and CO_2 , CH_4 and N_2O emissions in two contrasting soils (i.e. clayey and sandy tropical soils). It was tested the hypothesis that the combination of BC with vinasse and filter cake reduces GHG emissions to the atmosphere, mainly N_2O , while improving soil chemical attributes.

3.2 Material and Methods

Two incubation experiments were conducted under controlled environmental conditions (i.e. temperature and moisture), with and without application of vinasse and filter combined with addition rates of BC in two contrasting soils (i.e. clayey and sandy tropical soils).

3.2.1 Biochar, organic residues and soils

See Chapter 2 for detailed information regarding the BC production and characterization. Two soils with contrasting texture, a Quartzipsamment (sandy) and a Typic Hapludox (clayey), were collected from two different native vegetation areas located, respectively, near Anhembi town, State of Sao Paulo, Brazil (22° 43' 31.1" S and 48° 01' 20.2" W) and within the ESALQ/USP campus, Piracicaba, State of Sao Paulo, Brazil (22° 42' 05.1" S and 47° 37' 45.2" W). The soils were sampled at the 0-20 cm layer, air-dried, homogenized, and sieved to 2 mm. Chemical and physical characteristics of the soils used in the incubation experiments are given in Table 3.1.

The filter cake was collected fresh from a sugarcane mill located in Piracicaba, State of Sao Paulo, Brazil, and dried at 45°C for 48 hours in a forced-air oven. The dried material was gently crushed and sieved (< 2 mm) before incubation. The vinasse was directly collected from

the storage tanks and kept frozen at -20°C prior to application to the experimental units. Filter cake and vinasse characteristics are reported in Table 3.2.

Table 3.1 – Soil properties of the two incubated soils used to evaluate the combination of BC with organic residues from sugarcane industry

Soils	Sand / Silt / Clay (<i>g kg⁻¹</i>)	<i>pH</i> [†]	<i>C</i>	<i>N</i>	<i>C/N</i>	$\delta^{13}\textit{C}$	$\delta^{15}\textit{N}$
			%		‰		
Sandy	900 / 22 / 78	4.1 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	14.3 ± 0.1	-27.0 ± 0.1	8.3 ± 0.1
Clayey	406 / 277 / 317	6.2 ± 0.0	1.9 ± 0.1	0.2 ± 0.0	11.3 ± 0.2	-25.5 ± 0.0	7.9 ± 0.5

Mean $\pm$ standard deviation ($n = 3$).

$^{\dagger}pH$ in CaCl_2 .

Table 3.2 – Characterization of the organic residues (filter cake and vinasse) used in the incubation experiments

Residues	$pH^{\dagger}$	C	N	C/N	Ca	Mg	S	P	K
		$(g\ kg^{-1})$			$(g\ kg^{-1})$				
Filter cake	6.5	133.9	8.4	16	10.0	1.7	0.8	10.5	1.4
Vinasse	4.7	17.0	0.8	22	0.8	0.3	0.5	0.6	3.5

$^{\dagger}pH$ in CaCl_2 .

3.2.2 Experimental set-up and design

Two incubation experiments were performed, each with four treatments that comprised eight (8) replicates, following a complete randomized design, with destructive samples throughout the experiment (30, 60 and 100 days) to characterize soil chemical attributes over the time of incubation. The treatments of both experiments are shown in Table 3.3. The incubation units consisted of 200 g dry soil and 0.75, 1.5 and 4 g of BC (experiment with sandy soil) and 1, 2 and 5 g of BC (experiment with clayey soil). These additions represent field application rates of 10, 20 and 50 Mg ha^{-1} of BC, respectively (assuming an incorporation depth of 20 cm and a bulk density of 1.0 and 1.3 g cm^{-3} for clayey and sandy soil, respectively). These were then pre-incubated in a refrigerator overnight and placed in airtight glass jars of 1.4 liters at 25°C .

The amount of filter cake and vinasse applied to all treatments was equivalent to 100 Mg ha^{-1} and 100 $\text{m}^3 \text{ha}^{-1}$, respectively, which are the application rates commonly used in sugarcane fields in Brazil. Soil moisture was maintained by weighing the glass jars and adding necessary deionized water to bring the soils to 60% water-filled pore space ($WFPS$), considering the water holding capacity (WHC) of the BC.

Table 3.3 – Description of the treatments in the incubation experiments with sandy and clayey soils combined with BC and residues from the sugarcane industry

Treatments	Description
Control	Soil-alone [†]
T1	Soil + filter cake [‡] + vinasse [§]
T2	Soil + filter cake + vinasse + BIOCHAR 10 $Mg\ ha^{-1}$
T3	Soil + filter cake + vinasse + BIOCHAR 20 $Mg\ ha^{-1}$ [¶]
T4	Soil + filter cake + vinasse + BIOCHAR 50 $Mg\ ha^{-1}$ [#]

[†]200 g of soil (clayey or sandy) for each incubation unit.

[‡] mass equivalent to 100 $Mg\ ha^{-1}$; [§] volume equivalent to 100 $m^3\ ha^{-1}$.

^{||}0.75 g of BC for experiment 1 (sandy soil) and 1 g of BC for experiment 2 (clayey soil).

[¶]1.5 g of BC for experiment 1 and 2 g of BC for experiment 2.

[#]4 g of BC for experiment 1 and 5 g of BC for experiment 2.

3.2.3 Experiment evaluations

The fluxes of CO_2 , N_2O and CH_4 in each treatment were estimated by determining the concentration of the gases in the headspace of the jars between sampling events. After homogenization of the atmosphere within the jars, gas samples were collected using 20 mL syringes and the concentrations of CO_2 , N_2O and CH_4 were determined using a gas chromatograph. The results were used to estimate the fluxes of CO_2 ($mg\ CO_2 - C\ m^{-2}\ h^{-1}$), N_2O ($\mu g\ N_2O - N\ m^{-2}\ h^{-1}$) and CH_4 ($\mu g\ CH_4 - C\ m^{-2}\ h^{-1}$), based on the equation proposed by Clayperon (1834) (See Chapter 2).

For each experimental unit (jar), the total quantities of CO_2 , CH_4 and N_2O emitted during the evaluation period (cumulative emission) were estimated by mathematical integration of the curves of the daily fluxes. To assess the chemical characteristics of the incubated soils over time, destructive sampling events were performed 30, 60 and 100 days after the beginning of the incubations, by removing two (2) incubation units per treatment at 30 and 60 days, and the remaining replicates (4) were taken after 100 days. Subsamples were kept at $-20^\circ C$ for subsequent determination of mineral N (i.e. ammonium ($NH_4^+ - N$) and nitrate ($NO_3^- - N$)) by extraction (1:5 w:v) in a 1M KCl solution. Extracts were immediately frozen and kept for further measurement using the flow injection analysis method (RUZICKA; HANSEN, 1981).

The pH was determined following the methodology described by Anderson & Ingram (1989). The electrical conductivity (EC) was measured using a conductivity probe for a BC:solution ratio of 1:2 (w:v) suspended in ultrapure water. Available P and exchangeable cations (K^+ , Ca^{2+} , Mg^{2+}) were performed according to the methodology proposed by van Raij & Quaggio (1983). S content was determined by extraction in monocalcium phosphate 0.01M and subsequent quantification by colorimetry. The exchangeable acidity (Ac) was determined according to Embrapa (1997) by extraction of H^+ and Al^{3+} with a $Ca(OAc)_2$ 1.0M solution buffered to pH 7. Calculations of CEC at pH 7.0 (T) and base saturation ($BS\%$) were also performed.

3.2.4 Statistical analyses

A nested analysis of variance (Nested ANOVA) was carried out to the results regarding soil chemical attributes. The Tukey's test was applied for the comparison of mean values between and within treatments (over time). The mean cumulative GHG fluxes obtained for all treatments were also submitted to ANOVA and Tukey's test. All statistical analyses were run using the software R (R Core Team, 2014).

3.3 Results and Discussion

The Tables 3.4 and 3.5 show the chemical attributes of the incubated sandy and clayey soils, respectively, with different treatments evaluated over time. In general, the results indicate soil-specific BC interactions, since the effects of the evaluated treatments on soil chemical attributes were less pronounced in the incubation with clayey soil compared with the sandy soil.

Regarding the incubation with sandy soil, the *T4* presented the highest soil *pH* among treatments ($P < 0.05$) after 30 days of incubation, and *T1*, *T2* and *T3* did not differ ($P > 0.05$) (Table 8). Although the same trend was observed after 60 and 100 days of incubation (i.e. increased soil *pH* with BC application), the initial rise in soil *pH* values diminished between evaluation periods. However, this reduction was higher in *T1* compared with the other treatments, of 1.1 units after 100 days of incubation, while in *T2*, *T3* and *T4* ranged from 0.6 to 0.8 unit (Table 3.4).

Primarily, the quality of the feedstock and pyrolysis conditions have a direct impact on BC's *pH* (see Chapter 2). Increased soil *pH* levels after BC application have been attributed to its richness in alkaline cations such as Ca^{2+} , Mg^{2+} and K^+ , which are released through dissolution of the mineral phase to the soil solution, providing readily available nutrients for plant growth (GLASER; LEHMANN; ZECH, 2002; CHAN et al., 2008; IPPOLITO et al., 2015). The further reduction in soil *pH* may be an effect of the vinasse application, since all treatments evaluated had the same trend over time (Table 3.4).

The influence of vinasse on soil electrochemical attributes under controlled conditions was assessed by Ribeiro et al. (2012) that observed a decrease in soil *pH* 1 day after vinasse application to soil. As the incubation time increased, the *pH* increased as a function of the vinasse dose. However, after 60 days of incubation the *pH* values where vinasse was applied reached that of the control samples, indicating its transient effect on soil *pH*. McClellan et al. (2007) also observed that BC amendment increased soil *pH* temporarily, and as the biochar "aged" in the soil, the equilibration of alkaline species with carbon dioxide lowered its solution *pH*. However, this process required several wet/dry/leaching cycles to occur. Another interesting fact was the steeper decrease in *pH* values in *T1* compared with the *T2*, *T3* and *T4* (Table 3.4), which can be assigned to the real potential of pure BC to maintain increased soil *pH*, or its *pH* buffering capacity, reinforcing its role in improving soil acidic conditions.

Consequently, the increase in soil pH in the incubated sandy soil precipitated ions Al^{3+} by hydrolysis and decreased the Al^{3+} saturation of the exchange sites (Table 3.4), which often are major constraints for agricultural productivity in highly weathered soils of the humid tropics (COCHRANE; SÁNCHEZ, 1980; MBAGWU; PICCOLO, 1997). As expected, there were not significant changes in soil pH levels in clayey soil regarding all treatments and evaluation periods (Table 3.5), indicating that the extent of these changes will strongly depend on the pH -buffering capacity of the soil (TRYON, 1948; MUKHERJEE; LAL, 2014).

In relation to the available P in sandy soil, $T1$, $T2$ and $T3$ did not differ, and $T4$ presented the highest value among treatments ($P < 0.05$), resulting in a 72% increase in available P relative to $T1$ after 30 days of incubation (Table 3.4). There was a significant decrease in available P over time in all treatments, with $T1$ and $T4$ presenting P available 48% and 40% lower, respectively, after 90 days of incubation ($P < 0.05$). Nevertheless, $T4$ had the highest available P among treatments, about 2-fold higher than $T1$ (Table 3.4). The greater P availability in the first 30 days of incubation is a result of the initial rise in soil pH , which in turn influenced the availability of the P from the filter cake, a rich- P residue (see Table 3.2) with about 50% of its total P available in the short-term under favourable soil conditions (SPIRONELLO et al., 1997; CANTARELLA; ROSSETTO, 2008).

This is even more evident from the results found over time, as the decrease in this element is concomitant with the reduction of soil pH in all treatments. The result observed in $T4$ after 90 days is also due to the maintenance of soil pH with the highest BC dose (Table 3.4). In the case of most BCs, P will likely be associated with Ca and Mg , with some of these compounds in readily available forms (IPPOLITO et al., 2015). However, the total P present in the BC produced corresponds to a small fraction of the ash content (see Chapter 2), not being enough to promote a significant increase in soil available P by itself.

Regarding the contents of exchangeable basic cations in sandy soil, all treatments presented a significant increase in Ca^{2+} , Mg^{2+} and K^+ contents compared with the control ($P < 0.05$) (Table 3.4). Consequently, it was observed a decrease in the potential acidity (Ac) during incubation time ($P > 0.05$), showing that H^+ and Al^{3+} were displaced from the ion-exchange sites due to the greater concentration of exchangeable bases (NOVAK et al., 2009).

The $T2$, $T3$ and $T4$ presented the highest K^+ contents and differed from $T1$ ($P < 0.05$) in all evaluation periods (Table 3.4). In the first 30 days of incubation, the K^+ contents from $T2$ to $T4$ were 2 to 4 times higher relative to $T1$ (Table 3.4). Moreover, there was a slight increase in K^+ contents at day 100 relative to day 30 in all treatments with BC application. It was also observed higher Ca^{2+} and Mg^{2+} contents due to BC application in all evaluation periods, mainly for $T4$ ($P < 0.05$). Thus, the sum of bases (SB) was also significantly higher ($P < 0.05$) compared with the $T1$ (Table 3.4).

From these results it might be concluded that BC may act as a fertilizer itself, as most of the cations in the ash contained in BCs are not bound by electrostatic forces but in the form of dissolvable salts and, therefore, readily available for plant uptake (GLASER; LEHMANN; ZECH, 2002).

There was no clear pattern regarding the Ca^{2+} and Mg^{2+} contents, as well as P availability in the treatments evaluated in clayey soil. In the first 30 days of incubation, $T1$ presented a significant increase only in K^+ content, which was 127% higher compared with the control, and $T4$ presented K^+ content 18% higher than $T1$ ($P < 0.05$) (Table 3.5).

Tryon (1948) found increasing amounts of exchangeable bases after additions of 45% hardwood and conifer charcoals to sandy and loamy soils than in clayey soils. However, Rajkovich et al. (2012) observed that even on a fertile Alfisol corn growth significantly increased with greater P , K , and Mg contents in the BC, specially at high application rates of 7% by weight. Therefore, in some cases the mineral contents of BC play an important role for agronomic responses even on a fertile soil.

All treatments in sandy soil presented a significant increase in CEC in the first 30 days of incubation relative to the control ($P < 0.05$) (Table 3.4). After 90 days the CEC observed in $T2$, $T3$ and $T4$ was 43% to 59% higher compared with the $T1$ ($P < 0.05$), but the base saturation ($BS\%$) did not differ between the evaluated treatments ($P > 0.05$) (Table 3.4). In the incubation with clayey soil, higher CEC was observed in $T2$ in the first 30 days of incubation, which was 31% higher than $T1$ ($P < 0.05$). However, there were no differences among treatments in the subsequent evaluations (60 and 100 days) (Table 3.5). Generally, the impact of BC input in terms of CEC are more meaningful in sandy soils, while for many soils that already contain higher levels of organic matter and clay the impact of BC may be inconsequential (CLOUGH et al., 2013).

The results show that not only the nutrient contents but also the nutrient retention can be improved with BC additions to soil, especially in highly weathered soils with low ion-retention capacity (SCHULZ; GLASER, 2011). Mixing relatively large amounts of hardwood charcoal with soil increased the CEC by 50% compared with the unamended soil (TRYON, 1948; MBAGWU; PICCOLO, 1997). However, even low amounts of weathered charcoal have seen to increase CEC in soils Glaser (1999 apud GLASER; LEHMANN; ZECH, 2002).

The ability of some BCs to retain nutrients is attributed to its large surface area, porosity and acid surface functional groups that are expected to be negatively charged at likely soil solution pH conditions (MUKHERJEE; ZIMMERMAN; HARRIS, 2011). It is assumed that slow oxidation occurs on the edges of the aromatic backbone of BC, forming carboxylic groups responsible for the formation of organo-mineral complexes and thus the sustainably increased CEC (GLASER et al., 2000, 2001), by both biotic and/or abiotic processes.

According to Yoshizawa et al. (2005), the proliferation of microorganisms on the BC surface as well as between its pores leads to a slow oxidation process and produces carboxylic groups on the edges of the aromatic backbone, increasing the CEC . Also, the increasing pH with BC application promotes the progressive deprotonation of low pK_a functional groups on BC surface, which favours electrostatic surface charge neutralization by cations in the diffuse ion swarm. The low pK_a (≈ 4.5) of carboxyl functionality is responsible for the high CEC ($800 \text{ mmol}_c \text{ kg}^{-1}$) of BCs pyrolysed at $350 - 500^\circ\text{C}$ (HARVEY et al., 2012). Some ash

components, such as metal hydroxides and silica phytoliths can also provide negatively charged surfaces, although less than the carbonaceous matrix due to their higher point zero net charge ($PZNC$) (usually above 7) (HARVEY et al., 2011).

It should be highlighted that the saturation of the CEC by K^+ in sandy soil ranged from 19% ($T2$) to 29% ($T4$) by the end of incubation period, leading to an imbalance in the K^+ , Ca^{2+} and Mg^{2+} relationships in all treatments (Table 3.4). In Brazilian sugarcane production fields, the maximum dose of vinasse applied to soil is not likely to exceed the equivalent of 5% of K^+ in the CEC (CETESB, 2015), since extremely high K^+ may become soils saline, besides potentially displacing Ca^{2+} and Mg^{2+} from the exchange sites. Obviously, this complex dynamic must be carried out close to natural conditions, since the incubation units did not allow leaching and K^+ is a highly mobile element weakly retained by soil colloids, thus can be easily leached out from soil profile.

The high percentage of K^+ in the BC (see Chapter 2) and the increasing EC in $T1$ (11%), $T2$ (25%) and $T3$ (42%) relative to $T1$ in the first 30 days of incubation, indicate that the form of K^+ in BC is water-soluble, which continuously increased over time in all treatments ($P < 0.05$) (Table 3.4). As for the clayey soil, all treatments presented increased soil EC relative to control ($P < 0.05$), which is probably attributed to the vinasse applied to soil (PRADO; CAIONE; CAMPOS, 2013), but no differences were found from $T1$ to $T4$ ($P > 0.05$) in the first 30 days of incubation (Table 3.5). By the end of the incubation period, $T4$ presented a soil EC 17% higher than $T1$ ($P < 0.05$), which can be related to the higher K^+ content found with the highest application rate of BC, and $T1$, $T2$ and $T3$ were not different ($P > 0.05$) (Table 3.5). Cantrell et al. (2012) showed that total K^+ concentration was an important predictor of BC electrical conductivity, or the amount of salts present. Lehmann et al. (2003) found that higher K^+ in leachate after application of BC to soil, and attributed this to the high K^+ content of the BC itself.

Regarding the mineral N concentrations ($NH_4^+ - N$ and $NO_3^- - N$) in sandy soil, the treatments with BC additions initially presented lower $NH_4^+ - N$ and $NO_3^- - N$ in soil solution than the control and $T1$ BC ($P < 0.05$) (Table 3.4). In the first 30 days of incubation, the amount of $NH_4^+ - N$ in $T1$ was 43% higher than the control, and in $T2$ to $T4$ was 63% to 75% lower compared with the $T1$ (Table 3.4). The NO_3^- concentration in $T1$ was 20% higher relative to the control, and $T2$ to $T4$ was 50% to 65% lower relative to $T1$. After 60 and 100 days, there was a steep decrease in $NH_4^+ - N$ in all treatments, with concomitant increase in the amounts of $NO_3^- - N$, specially in $T2$ and $T3$ ($P < 0.05$), and $T4$ showed the lowest $NO_3^- - N$ among treatments ($P < 0.05$) (Table 3.4).

As for the mineral N forms in clayey soil, $T1$ presented the highest $NH_4^+ - N$ and $NO_3^- - N$ concentrations in soil solution, and $T2$, $T3$ and $T4$ did not differ in the first 30 days of incubation ($P > 0.05$) (Table 3.5). However, the $NH_4^+ - N$ and $NO_3^- - N$ concentrations found in $T1$ were significantly lower after 60 days of incubation, and the $NO_3^- - N$ concentrations with BC application increased by 59% ($T4$), 79% ($T3$) and 84% ($T2$), with no

differences between the evaluated treatments and the control ($P > 0.05$) (Table 3.5). After 100 days, there were also no differences between the evaluated treatments and the control regarding both mineral N forms.

The initially lower mineral N found in $T2$, $T3$ and $T4$ in both experiments may be attributed to the adsorption of both $NH_4^+ - N$ and $NO_3^- - N$ onto BC. Also, it has been suggested that physical entrapment in BC pore structures may be responsible for removing NH_4^+ from soil solution, which is an entirely possible mechanism given the diameter of the NH_4^+ ion (286 pm) (SPÄTH; KÖNIG, 2010) and the wide range of pore sizes in the BC produced (see Chapter 2). BC can sorb NH_4^+ through acid surface functional groups (e.g. carboxyl and hydroxyl) via cation exchange (LEHMANN et al., 2011; SPOKAS et al., 2012; ZHENG et al., 2013), thus reducing its availability for autotrophic conversion to NO_3^- for some period of time. As for the NO_3^- , its adsorption is more related to the presence of base functional groups and not a result of physical adsorption (KAMEYAMA et al., 2012).

One plausible explanation for the enhanced nitrification activity in $T2$ and $T3$ after 60 days of incubation, specially in sandy soil (Table 3.4), could be the lower ability of BC to retain NO_3^- compared with the NH_4^+ (JONES et al., 2012). Kameyama et al. (2012) concluded that NO_3^- was only weakly adsorbed onto BC and could be desorbed by water infiltration. Also, the stimulation of autotrophic nitrification in high pH microsites due to the liming effects of BC is a highly probable mechanism (NELISSEN et al., 2012). Recently, Nelissen et al. (2014) have found that nitrification was increased due to a greater NH_4^+ substrate supply for autotrophic nitrifiers. As the exact mechanism involved in adsorption of N forms onto BC surfaces and the effect of time on these processes remain to be understood, this may be a possible phenomenon.

The contrasting result found for $T4$ in particular may suggest that some microbial N immobilization had also been taking place, since the removal of NH_4^+ and NO_3^- from soil solution prevailed after 60 days compared with the $T2$ and $T3$ (Table 3.4). In this case, the highest BC dose may have contributed with a greater proportion of bioavailable C , such as residual bio-oils, resulting in microbial demand for inorganic N present in the soil solution (DELUCA; MACKENZIE; GUNDALE, 2009).

Generally, the abovementioned results indicated that, under the same experimental conditions the soil amelioration was closely related to its buffering capacity. In other words, the higher the soil CEC and its initial nutrient concentrations, the greater the soil buffering capacity and lower the effect of pure BC and its combination with organic residues on soil chemical attributes.

Figures 3.1 and 3.2 present the mean daily and cumulative fluxes of CO_2 , N_2O and CH_4 of the incubated sandy and clayey soils, with different treatments evaluated over time. As expected, there was an exponential decay in the mean daily CO_2 emissions over time regarding all treatments evaluated and both soil matrixes, with the lowest values observed for the soil-alone (Figure 3.1). In the incubated sandy soil, the application of filter cake and vinasse to soil resulted in cumulative CO_2 emissions 447% higher compared with the soil-alone (Figure 3.2).

Table 3.4 – Soil chemical attributes after 30, 60 and 100 days of incubation in a sandy soil matrix with sugarcane straw BC combined with residues from the sugarcane industry

	<i>pH</i>	<i>EC</i> $\mu S\ cm^{-1}$	<i>P</i> $mg\ dm^{-3}$	<i>S</i> $mg\ dm^{-3}$	<i>K</i>	<i>Ca</i>	<i>Mg</i>	<i>Al</i> ³⁺ $mmol\ c\ dm^{-3}$	<i>Ac</i>	<i>SB</i>	<i>CEC</i>	<i>BS</i> %	<i>Al</i> _{sat}	<i>NH</i> ₄ ⁺	<i>NO</i> ₃ ⁻ $mg\ kg^{-1}$	<i>N</i> _{min}
30 days																
C	4.3(0.1) Ac	127.6(4.42) Ce	8.0(0.0) Ac	10.0(0.5) Ab	1.6(1.2) Ad	3.3(2.3) Ac	3.3(0.6) Ac	3.7(1.0) A	15.6(1.5) Aa	8.2(3.3) Ad	23.8(2.7) Ad	34.4(1.5) Ac	18.3(3.7) C	17.3(0.0) Ab	51.9(1.0) Ab	69.2 (1.1) Ab
T1	6.3(0.1) Ab	576.4(18.4) Cd	53.6(3.2) Ab	79.0(0.0) Aa	3.8(0.7) Ac	22.7(1.7) Aab	7.0(0.8) Ab	0.0(0.1) A	5.3(1.8) Ab	33.5(2.3) Ac	38.8(2.2) Ac	86.3(3.4) Ab	0 C	24.7(2.8) Aa	62.5(1.2) Aa	87.2(1.0) Aa
T2	6.2(0.0) Ab	641.5(7.8) Cc	68.3(5.5) Ab	83.0(0.2) Aa	8.9(0.7) Bb	25.7(2.1) Aa	8.0(0.2) Aab	0.1(0.0) A	5.1(1.3) Ab	42.6(1.2) Abc	47.7(3.6) Ab	89.3(2.7) Aab	0 C	7.0(0.2) Abc	22.1(3.0) Bc	29.1(5.0) Bc
T3	6.2(0.0) Ab	718.7(15.4) Bb	67.0(16.5) Ab	85.0(0.0) Aa	12.0(0.9) Bb	26.3(2.0) Aa	8.0(0.0) Aab	0.1(0.0) A	5.1(0.6) Ab	46.3(1.5) Aa	51.4(1.2) Aa	90.1(1.5) Ba	0 C	9.2(1.9) Ab	27.9(5.0) Bc	37.1(5.6) Bc
T4	6.6(0.1) Aa	820.2(14.0) Ba	92.0(10.4) Aa	80.0(0.1) Ba	14.5(1.4) Ba	20.7(1.3) Ab	9.0(0.5) Aa	0.1(0.0) A	4.8(0.9) Ab	44.2(1.3) Aab	49.0(3.2) Aab	90.2(1.5) Aa	0 C	6.1(11.5) Ac	30.9(6.0) Ac	37.0(2.8) Bc
60 days																
C	4.2(0.0) Ad	185.9(5.1) Bd	12.3(1.2) Ac	11.0(0.0) Ac	2.0(0.7) Ad	3.3(1.5) Ad	2.7(0.6) Ab	2.0(0.6) C	13.7(1.7) Ba	8.0(2.4) Ad	31.7(2.8) Bc	25.2(4.0) Bc	21.7(4.0) B	6.3(0.1) Ba	46.2(1.0) Aa	52.5(1.2) Ab
T1	5.3(0.1) Bc	630.5(5.6) Bc	28.0(2.7) Bbc	74.0(0.0) Ab	3.4(0.7) Ac	12.7(1.5) Bc	6.7(0.0) Aa	< 1 A	6.0(0.6) Ab	22.8(1.3) Abc	28.8(2.0) Ac	79.2(1.0) Ab	0 C	4.5(0.5) Bb	52.7(3.2) Ba	57.2(3.0) Ab
T2	5.6(0.1) Bb	689.7(17.0) Bb	49.3(7.4) Ba	77.3(0.0) Bab	9.6(1.7) Ab	14.3(1.2) Cb	8.0(0.0) Aa	< 1 A	5.7(1.2) Ab	31.9(2.8) Bc	37.6(3.5) Bb	84.8(1.2) Bab	0 C	3.7(0.0) Bab	71.5(3.4) Aa	75.2(0.3) Ba
T3	5.7(0.1) Bb	707.4(13.0) Bb	37.0(12.5) Bab	83.3(0.0) Aa	12.3(2.0) Bab	19.0(1.2) Bab	7.3(1.0) Aa	< 1 A	5.7(0.6) Abb	38.6(3.7) Aab	43.3(3.1) Aab	89.1(2.0) Aa	0 C	3.0(0.9) Cc	61.7(3.7) Aa	64.7(3.2) Ba
T4	6.1(0.1) Ba	863.7(15.4) Aa	39.7(13.3) Cab	79.3(0.0) Bab	14.6(0.5) Ba	20.7(0.6) Aa	9.0(0.0) Aa	< 1 A	5.1(0.6) Ac	44.3(1.1) Ba	49.4(1.9) Ba	89.7(0.6) Aa	0 C	4.5(0.7) Bb	37.2(5.2) Ab	41.7(6.3) Bc
100 days																
C	4.1(0.0) Ad	252.7(8.2) Ad	7.0(1.5) Ac	10.7(1.0) Ad	1.3(0.2) Ad	3.7(0.6) Ac	3.0(0.6) Ab	3.0(2.0) B	16.0(1.1) Ba	8.0(0.4) Ad	24.0(0.5) Bc	33.3(0.6) Bc	28.7(0.6) A	1.2(1.6) Cc	56.9(3.9) Aab	58.1(3.1) Aab
T1	5.2(0.1) Bc	664.8(13.9) Ac	28.0(1.0) Bb	76.3(1.0) Ac	3.9(1.1) AC	15.0(1.5) Bb	6.7(1.5) Aa	< 1 A	6.0(6.1) Ab	25.6(0.2) Ac	31.6(0.9) Ab	81.0(1.1) Ab	0 C	1.0(2.2) Cc	49.5(10.1) ABb	50.5(9.7) Ab
T2	5.4(0.2) Cbc	748.9(22.1) Ab	40.7(1.5) Bab	83.7(0.6) Ab	10.3(0.1) Ab	21.0(0.6) Ba	8.0(1.0) Aa	< 1 A	5.8(1.1) Ab	39.3(1.2) Ab	45.1(1.7) Aa	87.1(0.6) Aab	0 C	1.9(1.9) Bbc	68.1(8.8) Aa	70.0(8.9) Aa
T3	5.6(0.2) Bab	792.1(15.8) Aa	35.7(2.9) Bb	85.7(0.6) Ab	13.6(1.6) Ab	20.3(1.0) Ba	7.7(0.6) Aa	< 1 A	5.7(0.6) Bb	41.6(2.7) Aab	47.3(0.2) Aa	87.9(0.5) Aab	0 C	4.1(2.4) Ba	61.1(9.0) Aa	65.2(13.3) Aa
T4	5.8(0.0) Ca	824.1(50.6) Ba	55.3(2.0) Ba	106.0(1.5) Aa	15.5(2.0) Aa	21.0(1.5) Aa	8.0(0.6) Aa	< 1 A	5.8(0.6) Ab	44.5(0.2) Aa	50.3(0.2) Aa	88.5(1.1) Aa	0 C	3.2(2.2) Bab	36.6(9.0) Ab	39.8(6.9) Ab

Mean $\pm$ sd ($n = 2, 30$ and 60 days; $n = 4, 100$ days). Mean followed by the same uppercase letter compare within treatments between periods. Means followed by the same lowercase letter compare between treatments within period. Means do not differ statistically at 5% probability by Tukey's test. The treatments are: (T1) soil + filter cake + vinasse; (T2) soil + filter cake + vinasse + BC $10\ Mg\ ha^{-1}$; (T3) soil + filter cake + vinasse + BC $20\ Mg\ ha^{-1}$; (T4) soil + filter cake + vinasse + BC $50\ Mg\ ha^{-1}$. The control (soil-alone) is "C". *EC*: electrical conductivity; *Ac*: potential acidity; *SB*: sum of bases; *CEC* cation exchange capacity; *BS*: base saturation; *Al*_{sat}: saturation by *Al*³⁺; and *N*_{min}: mineral N.

Table 3.5 – Soil chemical attributes after 30, 60 and 100 days of incubation in a clayey soil matrix with sugarcane straw BC combined with residues from the sugarcane industry

	<i>pH</i>	<i>EC</i> $\mu S\ cm^{-1}$	<i>P</i> $mg\ dm^{-3}$	<i>S</i> $mg\ dm^{-3}$	<i>K</i>	<i>Ca</i>	<i>Mg</i> $mmol_c\ dm^{-3}$	<i>Ac</i>	<i>SB</i>	<i>CEC</i>	<i>BS</i> %	NH_4^+	NO_3^- $mg\ kg^{-1}$	N_{min}
30 days														
C	6.5(0.1) Aab	290.4(18.2) Cb	28.0(1.4) Ac	9.6(0.7) Bb	4.0(0.1) Ac	96.5(2.1) Bb	20.0(0.0) Bc	12.0(0.0) Ca	120.5(2.2) Bc	132.5(2.2) Bc	90.9(0.0) Bc	36.5(13.1) Aa	89.2(17.6) Aa	125.7(47.0) Aa
T1	6.7(0.0) Aa	529.8(9.9) Ba	98.5(10.6) Aab	17.6(14.8) Bab	9.1(0.3) Aab	103.5(2.1) Bb	23.6(0.0) Bbc	5.0(0.0) Cb	136.2(2.5) Bbc	141.2(2.5) Bbc	96.5(0.0) Bb	46.9(19.2) Aa	125.7(58.9) Aa	172.7(97.8) Aa
T2	6.6(0.0) Aab	565.8(39.8) Ba	116.0(52.3) ABab	21.6(2.1) Ba	7.0(0.7) Abc	142.0(5.7) Ba	29.6(0.7) Cb	6.0(0.0) Cb	178.6(5.7) Ba	184.6(5.7) Ba	96.8(0.0) Aa	12.8(0.1) Ab	38.4(0.4) Bb	51.2(7.3) Ab
T3	6.5(0.1) Aab	566.9(16.0) Ba	131.5(62.9) Aa	19.5(2.1) Cab	8.6(0.2) ABab	105.0(1.4) Cb	28.0(0.7) Ba	6.0(0.0) Bb	141.6(1.6) Cb	147.6(1.6) Cb	95.9(0.0) Bb	10.5(1.1) ABb	46.8(0.9) Bb	57.3(5.7) Ab
T4	6.5(0.0) Ab	557.7(23.6) Ca	70.5(9.2) Abc	26.5(0.7) Ba	10.7(1.6) Aa	97.0(2.8) Bb	26.0(0.0) Abab	5.0(0.0) Bb	133.7(1.3) Bbc	138.7(1.3) Bbc	96.4(0.0) Cb	10.9(1.5) Ab	42.5(6.4) Bb	53.3(13.3) Ab
60 days														
C	6.3(0.1) Ba	446.7(22.0) Bb	20.5(0.7) Ab	21.5(3.5) Ac	4.6(0.2) Ab	178.0(8.5) Aa	20.0(0.0) Bc	10.0(0.0) Ba	202.6(8.7) Aa	212.6(8.7) Aa	95.3(0.0) Abc	24.4(3.9) Aa	82.1(12.4) Aa	106.5(20.4) Aba
T1	6.5(0.1) Ba	803.9(32.3) Aa	86.5(38.9) Aa	40.5(2.1) Ab	5.7(0.4) Bb	171.0(4.2) Aa	36.0(4.2) Aa	9.0(1.4) Bab	212.7(0.4) Aa	221.7(1.8) Aa	95.9(0.7) Aab	13.3(0.7) Aab	60.3(7.6) Bab	73.6(4.0) Ba
T2	6.4(0.1) Ba	826.1(34.4) Aa	70.5(9.2) Aab	40.0(0.0) Ab	5.4(0.5) Ab	163.0(5.7) Aab	32.5(2.1) Aa	6.0(0.0) Bb	200.9(8.3) Aa	206.9(8.3) Aab	97.1(0.0) Aa	11.9(0.2) Aab	70.6(1.6) Aa	82.5(5.6) Aa
T3	6.4(0.1) Aba	945.4(120.0) Aa	63.5(14.8) Bab	60.0(9.9) Aa	9.9(4.5) Aa	139.5(23.3) Bc	24.5(0.7) Cb	6.0(2.8) Bb	173.9(19.5) Bb	179.9(22.3) Bc	96.6(0.7) ABc	18.4(5.6) Aab	84.0(3.8) Aa	102.5(10.8) Aa
T4	6.5(0.1) Aa	824.2(81.5) Ba	69.0(25.5) Aab	56.0(7.1) Aa	11.0(5.7) Aa	148.0(11.3) Abc	24.5(4.9) Bb	6.0(1.4) Aab	183.5(10.5) Ab	189.5(12.0) Abc	96.8(0.0) Bbc	8.4(1.6) Ab	67.4(7.2) Aa	75.8(12.6) Aa
100 days														
C	5.8(0.1) Cb	463.6(192.4) Ac	20.0(1.4) Ab	21.0(7.1) Ac	3.5(0.9) Ac	166.0(5.7) Aa	27.0(5.7) Ac	12.5(2.1) Aa	196.5(0.9) Aab	209.0(3.0) Aa	94.0(0.7) Cc	1.6(0.2) Ba	79.5(4.0) Aa	79.4(3.4) Ba
T1	6.3(0.1) Ca	869.7(8.1) Ab	87.0(53.7) Aa	44.5(9.2) Ab	5.2(0.3) Bbc	164.0(0.0) Aa	36.0(1.4) Aa	6.0(1.4) Ab	205.2(1.7) Aa	211.2(0.3) Aa	97.2(0.7) Bb	2.1(0.3) Ba	64.5(9.2) Ba	66.6(10.2) Ba
T2	6.3(0.1) Ba	879.8(9.2) Ab	120.0(2.8) Aa	39.5(2.1) Ab	5.7(0.3) Ab	165.0(24.0) Aa	33.0(0.0) Aa	5.0(0.0) Abc	203.7(23.8) Aa	208.7(23.8) Aa	97.6(1.4) Bb	4.6(3.6) Aa	71.3(6.9) Aa	75.9(7.0) Aa
T3	6.3(0.0) Ba	893.5(25.3) Aab	81.5(24.7) Ba	45.5(0.7) Bb	6.1(0.2) Bab	160.5(12.0) Aab	32.0(1.4) Aab	5.3(1.4) Ac	198.6(13.2) Aa	203.6(14.6) Aa	97.5(0.0) Ab	2.0(0.4) Ba	85.8(15.4) Aa	87.8(16.8) Aa
T4	6.3(0.2) Ba	1016.9(18.5) Aa	102.0(58.0) Aa	56.0(2.8) Aa	6.9(0.3) Ba	146.0(9.9) Ab	28.0(1.4) Abc	5.3(0.0) Bd	180.9(8.2) Ab	186.2(8.2) Ab	97.1(0.0) Aa	2.8(1.1) Aa	58.3(1.3) Ba	61.1(1.8) Aa

Mean $\pm$ sd ($n = 2, 30$ and 60 days; $n = 4, 100$ days). Means followed by the same uppercase letter compare within treatments between periods. Means followed by the same lowercase letter compare between treatments within period. Means do not differ statistically at 5% probability by Tukey's test. The treatments are: (T1) soil + filter cake + vinasse; (T2) soil + filter cake + vinasse + BC $10\ Mg\ ha^{-1}$; (T3) soil + filter cake + vinasse + BC $20\ Mg\ ha^{-1}$; (T4) soil + filter cake + vinasse + BC $50\ Mg\ ha^{-1}$. The control (soil-alone) is "C". *EC*: electrical conductivity; *Ac*: potential acidity; *SB*: sum of bases; *CEC* cation exchange capacity; *BS*: base saturation; and N_{min} : mineral N.

Also, it was observed a significant increase in the cumulative CO_2 emissions for T_2 (4%) and T_3 (8%) compared with the T_1 ($P < 0.05$) (Figure 3.2). However, the T_4 presented a 11% decrease in cumulative CO_2 emissions and did not differ statistically from T_1 (Figure 3.2). The cumulative CO_2 emissions in clayey soil were 140% higher due to the application of filter cake and vinasse compared with the soil-alone. The T_2 presented the highest cumulative CO_2 emission among treatments, followed by T_4 and T_1 , which were 14% and 15% lower, respectively, and T_3 presented the lowest cumulative CO_2 emission among the evaluated treatments (Figure 3.2), showing no clear trend regarding the cumulative CO_2 emissions with BC application to clayey soil.

Organic fertilizers in liquid form, such as vinasse, have high contents of water and dissolved organic carbon (TEJADA; GONZALEZ, 2005), which can enhance soil respiration and thus CO_2 emission. Also, the higher cumulative CO_2 emission with the addition of 10 and 20 $Mgha^{-1}$ of BC in sandy soil could be attributed to a greater proportion of inorganic C (i.e. carbonates) (JONES et al., 2011) and/or residual bio-oils with increasing doses, which can be released from within the BC and emitted as CO_2 (STEINER et al., 2007; NELISSEN et al., 2012; CLOUGH et al., 2013). However, the treatment with the highest BC dose did not follow the same trend as it did not result in increased CO_2 emissions and did not differ statistically from the treatment without BC application (T_1) (Figure 3.2).

Regarding the mean daily N_2O fluxes, it was observed N_2O emissions peaks for T_1 and T_2 in the first 20 days of incubation, and after 60 days for T_1 in the incubated clayey soil, while for the sandy soil it was not observed emissions peaks, but an increase in mean daily N_2O fluxes after 40 days of incubation in all treatments evaluated compared with the soil-alone (Figure 3.1). The application of filter cake and vinasse to soil increased the cumulative N_2O emissions by 537% and 125% in sandy and clayey soil, respectively ($P < 0.05$) (Figure 3.2). Besides the high biochemical oxygen demand of vinasse, which causes a temporally reduced environment after its application to soil (LEAL et al., 1983), the high amounts of bioavailable C in this residue can fuel nitrification and denitrification processes.

Carmo et al. (2013) observed that the application of vinasse combined with N fertilizer resulted in higher cumulative N_2O emissions than the mineral fertilizer alone. Paredes et al. (2013) have also found higher N_2O fluxes in soils treated with vinasse, with the highest fluxes in the first 20 days of experiment.

Oliveira et al. (2013) also observed significantly higher N_2O emissions in the first days after vinasse application in burnt and unburnt sugarcane areas. The same authors concluded that the ferti-irrigation with vinasse reduced soil aeration and increased the availability of labile C to microorganisms, causing microsite of anaerobiosis due to a higher demand of O_2 and stimulating denitrification processes in soil.

However, the application of increasing BC doses reduced the cumulative N_2O emissions by 24% (T_2) to 34% (T_4) in sandy soil, and by 14% (T_2) to 56% (T_4) in clayey soil compared with the T_1 ($P < 0.05$) (Figure 3.2).

Figure 3.1 – Mean daily fluxes of CO_2 ($mg\ CO_2 - C\ m^{-2}\ h^{-1}$), N_2O ($\mu g\ N_2O - N\ m^{-2}\ h^{-1}$) and CH_4 ($\mu g\ CH_4 - C\ m^{-2}\ h^{-1}$) measured over the incubation period in clayey and sandy soil matrix (error bars are ± 1 SD, $n = 8$ until 30 days, $n = 6$ until 60 days and $n = 4$ until 100 days) The treatments are represented as: (T1) soil + filter cake + vinasse; (T2) soil + filter cake + vinasse + BC $10\ Mg\ ha^{-1}$; (T3) soil + filter cake + vinasse + BC $20\ Mg\ ha^{-1}$; and (T4) soil + filter cake + vinasse + BC $50\ Mg\ ha^{-1}$. The control treatment (soil-alone) is presented as “Soil”

Although BC from sugarcane straw is a *N*-depleted material with a high *C* : *N* ratio (see Chapter 2), which may support *N* immobilization, it is still uncertain if the BC provides sufficient bioavailable *C* that can serve as microbial substrates and consequently promote conversion of mineral *N* into organic *N*, thereby reducing N_2O emissions.

In this context, the mechanisms by which some BCS could induce mitigation of soil N_2O emissions remain elusive (ZWIETEN et al., 2009; CAYUELA et al., 2013; CLOUGH et al., 2013), and will most likely be a function of both the BC and soil properties and their interaction. Primarily, the properties of the feedstock from which the BC is produced, in particularly its chemical composition (e.g. bioavailable *N* content, ash content, acid neutralizing capacity, aliphatic to aromatic *C* ratio etc) and physical properties (e.g. surface area, particle size, sorption capacity etc), have a significant impact on N_2O emissions. Generally, woody and crop residue BCS are generally more effective at lowering N_2O emissions than BCS produced from other feedstocks, such as manure or poultry litter (ZWIETEN et al., 2014).

Also, BC application in soil may affect N_2O emissions by changing soil physical, chemical and biological properties, which lead to several biotic and abiotic mechanisms that, operating concurrently, control *N* mineralization-immobilization and nitrification or denitrification processes in soil. The increase in soil aeration, as a function of BC type and application rate, may decrease N_2O emissions by reducing anaerobic micro-sites that favour denitrification (YANAI; TOYOTA; OKAZAKI, 2007; QUIN et al., 2014), mainly in soils with higher clay contents. As discussed previously, BC may also tighten the soil *N* cycling through direct sorption of NO_3^- , NH_4^+ , organic *N* compounds, enzymes and gases, including N_2O , onto BC surface and within pores (KAMEYAMA et al., 2012; NELISSEN et al., 2012; PROMMER et al., 2014).

More recently, the generation of a redox potential within BC pores have been studied, since the nitrification and denitrification processes are well-established redox reactions (VERSTRAETE; FOCHT, 1997). According to some authors, BCS produced below $550^\circ C$ are complex semi-conductors that can be reversibly oxidized and reduced and can thus act as either (or both) a source or sink of charge in multiple soil redox reactions (ZHANG; PANT; LOGAN, 2011; JOSEPH et al., 2013).

BCS are generally reduced (OH; SON; CHIU, 2013) and have an acid neutralizing capacity; thus its application in soil can result in a local increase in *pH* and decrease in redox potential (*Eh*), which together can promote changes in the denitrifying community. Cayuela et al. (2013) found a consistent decrease in N_2O/N_2 ratio after BC application and hypothesized that BC lowers N_2O emissions through enhanced completion of denitrification (i.e. the reduction of N_2O to N_2) by acting as an “electron shuttle” that facilitates the transfer of electrons to soil denitrifying microorganisms. Other studies have found that catalytic reactions between metal ions present in BC surface and either NO_3^- and NO_2^- to produce N_2 are energetically favourable in anaerobic conditions (FANNING, 2000).

Figure 3.2 – Mean cumulative fluxes of CO_2 ($mg CO_2 - C m^{-2} h^{-1}$), N_2O ($\mu g N_2O - C m^{-2} h^{-1}$) and CH_4 ($\mu g CH_4 - C m^{-2} h^{-1}$) measured over the incubation period in clayey and sandy soil matrix (error bars are ± 1 SD, $n = 8$ until 30 days, $n = 6$ until 60 days and $n = 4$ until 100 days) The treatments are represented as: (T1) soil + filter cake + vinasse; (T2) soil + filter cake + vinasse + BC 10 $Mg ha^{-1}$; (T3) soil + filter cake + vinasse + BC 20 $Mg ha^{-1}$; and (T4) soil + filter cake + vinasse + BC 50 $Mg ha^{-1}$. The control treatment (soil-alone) is presented as “Soil”

Additionally, the formation of biofilms on the surface of BC may lead to the oxidation of organic matter and CH_4 and transfer of the electrons produced from these reactions to the BC if the environment is anoxic, affecting the rate of reduction of N_2O to N_2 (YUAN et al., 2013). Finally, the mean daily and cumulative CH_4 fluxes observed in this study were negligible compared with the existing literature, indicating that there were no significant effects of the treatments evaluated and soil matrixes on net CH_4 fluxes (Figures 3.1 and 3.2).

3.4 Conclusion

1. The combination of biochar with filter cake and vinasse presented synergistic effects on soil chemical properties, mainly regarding soil acidity, cation exchange properties, available P and exchangeable bases contents;
2. The effects of this combination on soil amelioration were more evident in sandy soils than in clayey soils, suggesting soil-specific biochar interactions and the use of biochar not only as a soil conditioner, but also as a fertilizer itself in nutrient-poor tropical soils; and
3. The biochar produced in this study successfully suppressed N_2O emissions in contrasting soils treated with vinasse and filter cake, as a function of biochar application rate.

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4 THE ROLE OF BIOCHAR ON THE MITIGATION OF NITROUS OXIDE (N_2O) EMISSIONS AND EFFICIENCY OF NITROGEN FERTILIZATION

Abstract

Nitrogen (N) is crucial for crop productivity. However, the increased fertilizer consumption and N losses to the environment increase cultivation costs, threaten water supplies and generates greenhouse gases (GHG) emissions. In this context, the application of BC combined with synthetic fertilizers has been proposed for enhancing N availability while reducing N_2O emissions. Thus, this study aims to evaluate the effects of sugarcane straw BC on soil chemical attributes, N gaseous loss as N_2O and N use efficiency (N derived from the fertilizer – $Ndf f$, and recovery of ^{15}N – % R). It was conducted a greenhouse pot experiment with wheat cultivation in a Quartzpissament using a N -labelled source ($NH_4 [^{15}N]O_3$). The following treatments were evaluated: ($T1$) Soil, with N , no BC; ($T2$) Soil, with N , BC 10 $Mg ha^{-1}$; ($T3$) Soil, with N , BC 20 $Mg ha^{-1}$; ($T4$) Soil, with N , BC 50 $Mg ha^{-1}$; and a control treatment. BC decreased N_2O emissions from N fertilization from 59% ($T2$) to 63% ($T4$) compared to only N fertilizer. 35 days after planting, $T4$ presented a significant increase in soil pH and available P 39% and 30% higher than control and $T1$, respectively, which remained higher at harvest. Potential acidity (Ac) decreased by 7% ($T2$) and 26% ($T4$) relative to $T1$, and base saturation (BS) increased by 17% relative to $T1$. It was observed higher $NH_4^+ - N$ concentrations in $T1$ and $T2$, while $T3$ and $T4$ showed the lowest $NH_4^+ - N$. Additionally, $T4$ presented the highest $NO_3^- - N$ concentration among treatments. $T4$ had higher tillering and grain yield 27% higher than $T1$. Also, $T2$ to $T4$ had higher 100-grain weight and shoot biomass. $T3$ and $T4$ presented the highest $Ndf f$ in grains; $T2$ and $T3$ had the highest $Ndf f$ in the entire plant. Consequently, the treatments with BC had the lowest $Ndf f$ in soil. There were no differences regarding ^{15}N loss among treatments. The application of sugarcane straw BC to soil improves N availability and the efficiency with which N is acquired by the plant and converted to grain yield, enhancing crop performance. In addition, the BC amendment significantly reduced N_2O emissions that result from the application of nitrogen fertilizer to soil.

Keywords: Crop yields; Greenhouse gases; Isotope Ratio Mass Spectrometry; Pyrogenic carbon; Wheat; ^{15}N tracer method

4.1 Introduction

The intensification of agriculture and changes in cropping systems since the “Green Revolution” have led to a significant rise in agricultural production (LASSALETTA et al., 2014a). Nitrogen (N) is required by plants in the largest quantity and is most frequently the limiting factor in crop productivity. However, studies have shown significant losses of N fertilizer to the environment, increasing cultivation costs, threatening water supplies and generating greenhouse gases (GHG) emissions (VENTEREA et al., 2012; LASSALETTA et al., 2014b). Along with these consequences, fertilizer consumption is continuously increasing,

which is often not profitable since the additional crop yield produced with each successive unit of fertilizer becomes smaller.

In this context, identifying strategies to enhance nutrient use efficiency, mainly N , will assume more importance than the quantity of nutrients applied, becoming an emerging trend in agricultural management. One of the major reasons for low N use efficiency is the difficulty of synchronizing its application with crop N demands (RIBAUDO et al., 2011). Additionally, the application of mineral fertilizers directly increases the available N for nitrification and denitrification, thus increasing N_2O emissions. Organic amendments can also lead to the mineralization of the labile C pool, which in turn stimulates microbial activity and consequently increases N_2O emissions (CHADWICK et al., 2000). However, applying N fertilizer in combination with BC has been proposed for improving temporal synchrony between crop- N demand and soil- N availability (CAYUELA et al., 2013), enhancing N use efficiency and reducing environmental impacts, such as N loss by leaching and N_2O emissions (SPOKAS et al., 2012; VENTEREA et al., 2012; STAVI; LAL, 2012).

Nitrification is the aerobic oxidation of ammonium (NH_4^+) to nitrate (NO_3^-), while denitrification is the sequential reduction of NO_3^- to NO_2^- , NO , N_2O , and N_2 , which may be complete (N_2 as a final product); or incomplete, leading to intermediate volatile N , especially N_2O . Understanding the contribution of these two processes for N_2O emissions is important to develop effective mitigation strategies, since such gas has a global warming potential (GWP) ca. 300 times higher than CO_2 (IPCC, 2007) and it is highly sensitive to environmental factors and soil management practices. Soil characteristics such as pH , labile carbon, soil texture, water content and temperature affect biological activity and gas diffusion, thereby increasing or decreasing emission of $N_2 - N$ and $N_2O - N$ to the atmosphere.

BC is a C -rich material obtained from the thermal decomposition of biomass (e.g. wood, manure, crop waste, etc.) in an O_2 -free atmosphere, a process called pyrolysis. During this process, C and N are rearranged to aromatic and heterocyclic structures, which reduces its lability, potentially mitigating N_2O production by biotic mechanisms (ZWIETEN et al., 2014). Other BC properties such as porosity, specific surface area, pH , acidic/basic functional groups and redox properties vary with the feedstock and pyrolysis conditions and can alter N_2O production (SINGH et al., 2010; ENDERS et al., 2012). In addition, the interaction of BC and soil types can influence C and N mineralization, pH , soil aeration and activity and composition of soil microbes (LEHMANN et al., 2011; CLOUGH et al., 2013).

The interference of BC in the N cycle has been addressed in recent works. A meta-analysis using 30 research peer-reviewed articles published between 2007-2013 revealed a 54% reduction in N_2O emissions due to BC application to soil (CAYUELA et al., 2014). However, some studies show no difference or an increase in N_2O emissions (SAARNIO; HEIMONEN; KETTUNEN, 2013; SUDDICK; SIX, 2013; SÁNCHEZ-GARCÍA et al., 2014). In this sense, the mechanisms underlying N_2O emissions due to BC application to soil remain elusive (MUKHERJEE; LAL, 2014) and currently there is no information on this topic in

Brazil. The aim of the present study was to evaluate the effect of sugarcane straw BC on the mitigation of N_2O emissions and use efficiency of nitrogen fertilization.

To do so, a greenhouse pot experiment was conducted under wheat cropping to estimate the efficiency of nitrogen fertilization through the ^{15}N -tracer method from the application of N -labelled fertilizer ($NH_4 [^{15}N]O_3$) to soil, and to determine crop yields and N_2O emissions with application rates of BC. It was tested the hypothesis that BC reduces N_2O emissions to the atmosphere, while increasing the availability and use efficiency of N by plants, thereby increasing crop yields.

4.2 Material and Methods

A greenhouse pot experiment was conducted under wheat cropping with and without application of ^{15}N -labelled fertilizer and addition rates of sugarcane straw BC to a sandy tropical soil. The wheat crop was chosen because it is nitrogen-demanding and ease to be grown in pots.

4.2.1 Experimental set-up and design

The greenhouse pot experiment consisted of the following treatments: (Control) Soil, no N , no BC; ($T1$) Soil, with N , no BC; ($T2$) Soil, with N , BC 10 Mg ha^{-1} , ($T3$) Soil, with N , BC 20 Mg ha^{-1} ; and ($T4$) Soil, with N , BC 50 Mg ha^{-1} . The experimental design was a randomized complete block and consisted of twelve replicates (blocks) and five treatments per block, totaling 60 plots (pots).

4.2.2 Pots and chambers

The pots were made of PVC pipe (20 cm diameter and 20 cm length each pot). To ensure chamber sealing, zinc gutters were attached to the PVC pipes and the insulation between the internal and external environment was made by adding water to the gutters (Figure 4.1). The static chambers were also made of PVC pipe with 20 cm diameter and 25 cm length, with internal 12V coolers connected to batteries to ensure air convection within the chambers during the gas sampling.

4.2.3 Soil, biochar and N fertilizer

The soil used was a Quartzipsamment collected from a native vegetation area in the 0-20 cm layer, which is the same sandy soil used in the incubation experiment of Chapter 3. Before installing the experiment, the soil was air-dried, homogenised, and sieved to 2 mm. The chemical characteristics of the soil are described in Table 3.1 (Chapter 3). The BC used was produced from sugarcane straw pyrolysed at $450^\circ C$ and sieved to 2 mm prior application to soil.

Figure 4.1 – Pot design (A); Chamber design (B); and Coupled pot and chamber (C)

See Chapter 2 for detailed information regarding the characteristics of the feedstock, pyrolysis process and characterization of the BC. The nitrogen source used was ammonium nitrate ($NH_4 [^{15}N]O_3$) with a ^{13}C atom fraction of 4.92%.

4.2.4 Management

Each pot consisted of 8.2 kg of soil (simulating a 0-20 cm soil layer and bulk density of 1.3 g cm^{-3}) and BC incorporated at rates of 10 (31.4 g); 20 (62.8 g) and 50 (157 g) $Mg \text{ ha}^{-1}$ to the treatments T_2 , T_3 and T_4 , respectively. The crop used was wheat (*Triticum aestivum* L.) (Variety IAC 385 MOJAVE) and the experiment was led to the complete crop cycle (≈ 120 days). A density of ten (10) plants per pot was used for the wheat crop and roughing 7 days after plant emergence, leaving four (4) plants per pot.

The material added to each pot was insulated with a thick plastic bag, ensuring that soil, BC and/or the fertilizer added was not lost from the pots. Soil moisture was maintained by periodically weighing the pots and adding necessary deionized water to bring the soils to 60% water-filled pore space ($WFPS$), considering the water holding capacity (WHC) of the BC.

NPK fertilization at planting and split applications were performed following the “Recommendations for Fertilizer and Lime to the State of Sao Paulo” (CANTARELLA; RAIJ; CAMARGO, 1997), as described in Table 4.1. The isotopically N -labelled fertilizer was completely dissolved in deionized water and applied to the moist soil by hand pipettes. $CaCO_3$ and $MgCO_3$ A.C.S was used in a 3:1 ratio to correct soil pH . The lime requirement was calculated according to the soil base saturation, in order to reach $BS = 65\%$, which is the requirement for wheat cropping. The lime was incorporated vigorously and the soil was irrigated to 60% $WFPS$, allowing to react for 15 days.

Table 4.1 – Application rates of fertilizers at planting and split applications in the greenhouse pot experiment with and without ^{15}N -labelled fertilized and rates of BC

Time	$\text{N}^{\S}$	$\text{K}_2\text{O}^{\P}$	$\text{P}_2\text{O}_5^{\#}$	$\text{S}^{\P}$	B^a
Application rates (kg ha^{-1}) †					
Planting	30	30	90	10	1.0
2 nd split fertilization ‡	45	30	..	..	..
3 rd split fertilization $^{\text{II}}$	45	..	..	..	..
Total	120	60	90	10	1.0

† Application rates following the “Recommendations for fertilizer and lime to the State of Sao Paulo”.

‡ 30 – 40 days after plant emergence.

$^{\text{II}}$ 60 – 70 days after plant emergence.

§ ^{15}N -labelled ammonium nitrate ($\text{NH}_4 [^{15}\text{N}]\text{O}_3$).

¶ Potassium sulphate (K_2SO_4) at planting and Potassium chloride (KCl) at 1st split application.

$^{\#}$ Triple superphosphate (TSP).

^{a} Boric acid (H_3BO_3).

4.2.5 Experiment evaluations

4.2.5.1 Gas sampling

The fluxes of N_2O in each treatment were estimated by determining the concentration of the gases in the headspace of the chambers. The average height of each chamber, and hence the headspace volume, was calculated from the mean of five measurements taken from the soil surface to the middle of each of the four sides and one central point within the chamber. Gas sampling events were performed during one week following N fertilization at planting, first and second split applications. After homogenization of the atmosphere within the pots, gas samples were collected at 0, 15, 30 and 45 minutes after closure of the chambers using 20 mL syringes, and placed in pre-evacuated 12 ml vials prior to analysis (Figure 4.2).

The concentrations of N_2O were determined using a gas chromatograph. The results were used to estimate the fluxes of N_2O ($\mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$) based on the equation proposed by Clayperon (1834) (See Chapter 2). For each experimental unit (pot), the total quantity of N_2O emitted during each evaluation period (cumulative emission) was estimated by mathematical integration of the curves of the daily fluxes.

4.2.5.2 Soil chemical attributes

To assess the chemical characteristics of the soils with different treatments over time, destructive sampling events were performed 35 after plant emergence, by removing six (6) pots per treatment, and the remaining replicates (6) were taken at the end of the experiment (120 days). Subsamples were kept at -20°C for subsequent determination of mineral N (i.e. ammonium ($\text{NH}_4^+ - \text{N}$) and nitrate ($\text{NO}_3^- - \text{N}$)).

The pH was determined following the methodology described by Anderson & Ingram (1989). Available P and exchangeable cations (K^+ , Ca^{2+} , Mg^{2+}) were performed according

to the methodology proposed by van Raij & Quaggio (1983). *S* content was determined by extraction in monocalcium phosphate 0.01 *M* and subsequent quantification by colorimetry.

The exchangeable acidity (*Ac*) was determined according to Embrapa (1997) by extraction of H^+ and Al^{3+} with a $Ca(OAc)_2$ 1.0 *M* solution buffered to *pH* 7. Calculations of *CEC* at *pH* 7.0 (*T*) and base saturation (*BS%*) were also performed. Mineral *N* content of the treatments was determined at 30, 60 and 100 days by extraction (1:5 w:v) in a 1 *M KCl* solution. Extracts were immediately frozen and kept for further measurement using the flow injection analysis method (RUZICKA; HANSEN, 1981).

Figure 4.2 – Gas sampling (A) and general view of the greenhouse pot experiment (B)

4.2.5.3 Yield components and *N* use efficiency

The wheat growth and yield in each treatment were evaluated by measuring the plant height, dry weight of below (roots) and aboveground parts (shoots and grains), 100-grain weight and number of tiller, head and spikelets. Also, the total *N* content and ^{15}N in atom fraction was determined in subsamples of all plant parts, soil and BC within the pots of each treatment.

For dry weight determination, plants were separated at harvest into roots, shoots and grains, and dried at 50°C to constant weight. For total *N* and ^{15}N determinations, the oven-dried shoots and roots samples were ground in a Wiley mill, the grains were ground in an analytical mill and the soil was passed through a ball mill. Then, the samples were sieved to 100 mesh size and analyzed using a Isotope Ratio Mass Spectrometer coupled to a *CNS* elemental analyzer. The nitrogen derived from the $NH_4 [^{15}N]O_3$ (*Ndff*) was calculated by the following equation:

$$Ndff = \left[\frac{(a - c)}{(b - c)} \right] \times N_t, \quad (5)$$

where:

a is ^{15}N atom fraction in the sample (plant, soil and BC);

b is ^{15}N atom fraction in the fertilizer;

b is ^{15}N atom fraction in the international standard (ambient air); and
 N_t is total N content (mg per pot) in the sample (plant, soil and BC).

The N percentage recovery ($\%R$) from the $\text{NH}_4[^{15}\text{N}]\text{O}_3$ in the plant, soil and BC was calculated as:

$$\%R = \left(\frac{Ndf f}{D} \right) \times 100, \quad (6)$$

where:

D is dose of applied N (mg per pot).

The percentage of ^{15}N loss via gaseous emission was calculated as follows:

$$^{15}\text{N}_{\text{loss}} = 100 - \%R_{\text{plant}} - \%R_{\text{soil}} - \%R_{\text{BC}} \text{ (if applied)}, \quad (7)$$

4.2.6 Statistical analyses

ANOVA was carried out to the results regarding soil chemical attributes. The Tukey's test was applied for the comparison of mean values between and within treatments (over time). ANOVA and Tukey's test was also applied for wheat yields, $Ndf f$, $\%R$ and N_2O fluxes. Pearson correlation was performed for $\%R$ in plants and soil, ^{15}N loss, crop productivity and some soil properties. All statistical analyses were run using R (R Core Team, 2014).

4.3 Results and Discussion

Figures 4.3 and 4.4 present the mean daily and cumulative fluxes of N_2O , respectively, measured over the experiment period for each treatment evaluated. Figure 4.5 presents the mean cumulative fluxes of N_2O expressed in CO_2 equivalent. The highest mean daily N_2O fluxes occurred during the first week after planting. At day 1, N_2O fluxes were significantly higher when N fertilizer was applied without BC ($T1$) ($442.71 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$), and decreased as a function of BC dose, from $T2$ ($152 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$) to $T4$ ($18 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$) ($P < 0.05$) (Figure 4.3). After day 3, N_2O fluxes decreased strongly to $< 100 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$ in all treatments, and $T2$ to $T4$ did not differ from the control treatment ($P > 0.05$).

After the 2nd N split application (days 36 to 42), mean daily N_2O fluxes were $< 30 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$. It was observed statistical differences between treatments only 1 day after N fertilization (day 36), in which $T2$ presented daily N_2O emission 45% higher ($22 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$) compared with the $T1$ ($12 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$). Also, emission peaks of N_2O were observed after 3rd N split application at days 72 and 73, for $T2$ and $T1$, respectively, differing from the other treatments evaluated ($P < 0.05$) (Figure 4.3).

The highest cumulative N_2O fluxes regarding the N fertilization at planting (1 to 7 days) were observed for $T1$ ($666 \mu\text{g N}_2\text{O} - \text{N m}^{-2} \text{h}^{-1}$) ($P < 0.05$). $T2$ to $T4$ did not differ, and

showed average N_2O emissions 75% lower compared with the $T1$ ($P < 0.05$) (Figure 4.4). The control treatment showed the lowest N_2O emissions among treatments ($P < 0.05$).

On average, the N_2O emissions from the 1st (day 36 to 42) and 2nd (day 69 to 75) N split applications were lower compared with the N fertilization at planting. From day 36 to 42, the highest cumulative N_2O emissions were also seen for $T1$ ($86 \mu g N_2O - N m^{-2} h^{-1}$) ($P < 0.05$), and $T2$ to $T4$ did not differ, showing average N_2O emissions 31% lower compared with the $T1$ ($P < 0.05$). The control treatment showed the lowest N_2O fluxes ($P < 0.05$) (Figure 4.4). From day 69 to 75, the cumulative N_2O fluxes were not different between the treatments evaluated ($P > 0.05$), and the control also showed the lowest emissions ($P < 0.05$). Taking together the results obtained from the three sampling events, increasing doses of BC decreased N_2O emissions from N fertilizer by 59% ($T2$) and 63% ($T4$) compared to only N fertilizer (Figure 4.4).

Considering the contribution of N_2O to global warming, the BC-containing treatments resulted in N_2O emissions converted into CO_2 equivalents 73% to 78% lower when compared with mineral N fertilizer only ($P < 0.05$) and did not differ from the control, relative to the first N split application (Figure 4.5). There were no significant differences between treatments regarding the 2nd and 3rd N split applications ($P > 0.05$) (Figure 4.5).

The highest N_2O emissions at day 1 are most likely a direct consequence of the initial fertilizer application and soil moisture adjustment (HARTER et al., 2014). The significantly lower N_2O emissions in the BC-amended treatments observed within the first week are agree with previous field and laboratory investigations using different BCs and soils (YANAI; TOYOTA; OKAZAKI, 2007; SINGH et al., 2010; ZWIETEN et al., 2010; TAGHIZADEH-TOOSI et al., 2011; ZHENG et al., 2013; CAYUELA et al., 2013; SÁNCHEZ-GARCÍA et al., 2014; HARTER et al., 2014; PROMMER et al., 2014; QUIN et al., 2014).

According to these studies, the most important environmental factors responsible for the reduced N_2O emissions with BC application are: (i) limited bioavailability of electron donors and acceptors (DOC , NO_3^- and NH_4^+) for microbial nitrification and denitrification due to sorption/immobilization onto BC particles (SINGH et al., 2010; TAGHIZADEH-TOOSI et al., 2011; ZHENG et al., 2013; PROMMER et al., 2014); (ii) improved soil aeration through BC addition and consequently reduced denitrification (YANAI; TOYOTA; OKAZAKI, 2007; ZWIETEN et al., 2010; QUIN et al., 2014); and (iii) increased abundance of nitrous oxide reductase (*nosZ*) in soil due to elevated soil pH values caused by BC addition (ZWIETEN et al., 2010; CAYUELA et al., 2013; HARTER et al., 2014).

The primary challenge for N_2O mitigation is directly associated with its high global-warming potential (FORSTER et al., 2007). Thus, a negligible amount of N_2O loss from an agronomic perspective can represent a substantial amount from a GHG perspective (VENTEREA et al., 2012). The lower CO_2eq emissions in BC-amended soils represent the real potential of BC as mitigating agent of GHG emissions to the atmosphere. Table 4.2 present the soil chemical attributes after 35 days (before 2nd N split application) and 120 days

Figure 4.3 – Mean daily fluxes of N_2O measured over the experiment period in sandy soil matrix (error bars are ± 1 SD, $n = 12$ at first evaluation and $n = 6$ at second and third evaluations). The treatments are represented as: (T1) Soil, with N, no BC; (T2) Soil, with N, BC 10 Mg ha^{-1} ; (T3) Soil, with N, biochar 20 Mg ha^{-1} ; and (T4) Soil, with N, biochar 50 Mg ha^{-1} . The control treatment (Soil, no N, no BC) is presented as “Control”. Panels 1, 2 and 3 represent each gas sampling event after N application to soil, corresponding to 1st split N application at planting (1), 2nd split N application (2) and 3rd split N application (3)

(harvest) of experiment. 35 days after plant emergence, T4 presented a small but significant increase in soil pH , 0.5 and 0.3 unit higher than control and T1, respectively ($P < 0.05$). The same treatment also presented the highest available P among treatments, 39% and 30% higher than control and T1, respectively ($P < 0.05$), and 28% higher than the T2 (Table 4.2).

Regarding the contents of exchangeable bases, increasing rates of BC application significantly increased soil K^+ contents, which were 3 (T2) to 8.5 (T4) times higher than T1 ($P < 0.05$) after 35 days of experiment, and no differences were observed in Ca^{2+} and Mg^{2+} contents among treatments ($P > 0.05$). At harvest, the available K^+ remained higher in T4 compared to the other treatments ($P < 0.05$). As a consequence of the higher K^+ content, potential acidity (Ac) decreased by 7% (T2) and 26% (T4) relative to T1 ($P < 0.05$), and base saturation (BS) increased by 17% relative to T1. No significant effects of the evaluated treatments on SB and CEC were observed ($P > 0.05$).

Also, no available Al^{3+} was found in all treatments (data not shown). In relation to the mineral N forms, there was no statistical differences in both $NH_4^+ - N$ and $NO_3^- - N$ concentrations among treatments after 30 days of experiment. However, by the end of the experiment, it was observed higher concentrations of $NH_4^+ - N$ in T1 and T2 ($P < 0.05$), while the control, T3 and T4 showed the lowest $NH_4^+ - N$ concentrations. Additionally, T4 presented the highest $NO_3^- - N$ concentration, differing from the other treatments ($P < 0.05$). Although the recalcitrant nature of BC makes it unlikely to be a balanced fertilizer, its addition to soil plays a key role on the dynamics of nutrients, both by direct addition and indirectly by

Figure 4.4 – Mean cumulative fluxes of N_2O measured over the experiment period in sandy soil matrix (error bars are ± 1 SD, $n = 12$ at the first evaluation and $n = 6$ at the second and third evaluations). The treatments are represented as: (T1) Soil, with N, no BC; (T2) Soil, with N, BC 10 Mg ha^{-1} ; (T3) Soil, with N, BC 20 Mg ha^{-1} ; and (T4) Soil, with N, BC 50 Mg ha^{-1} . The control treatment (Soil, no N, no BC) is presented as “C”. Panels 1, 2 and 3 represent each gas sampling event after N application to soil, corresponding to 1st split N application at planting (1), 2nd split N application (2) and 3rd split N application (3)

affecting soil properties that drive the availability of nutrients. BC-induced changes in soil nutrient conditions particularly affect the cycling of P and K (RAJKOVICH et al., 2012). Both nutrients may be directly introduced to the soil through either labile organic compounds and minerals in the ash fraction of BC and become available as these compounds weather (YAMATO et al., 2006).

As discussed in the previous chapter, the liming effect of BC reduces the concentration of dissolved ions that interact with $H_2PO_4^-$ in low- pH soils, such as Fe^{3+} and Al^{3+} , so the previously bound P then becomes better available to plants (XU et al., 2014). Moreover, as pH increases the soil surface becomes more negatively charged, thus increasing anion repulsion and decreasing P sorption by soil colloids (HAYNES, 1982).

Potassium typically concentrates in BC and tends to be highly available (LEHMANN et al., 2003; IPPOLITO et al., 2015). It is important to point out that the supply of K to plants stimulates the efficient use of N through plant uptake and assimilation, consequently increasing crop yield (VIANA; KIEHL, 2010). Assuming a 50% availability of the total K present in BC (12.46 mg kg^{-1}) and the 60 kg ha^{-1} of K_2O required by the wheat crop, about 8 Mg ha^{-1} of BC would be applied to meet the crop K demands.

Decreasing NH_4^+ concentrations with increasing BC dose could either be due to higher NH_4^+ assimilation (HARTER et al., 2014) or by increased oxidation of NH_4^+ to NO_2^- and then NO_3^- . The highest NO_3^- concentration with the application of 50 Mg ha^{-1} of BC is also an indicative of the processes above related. This is important because nitrification acts as a key process in determining fertilizer use efficiency in crops as well as N losses because it not only

Figure 4.5 – Mean cumulative fluxes of N_2O measured over the experiment period in sandy soil matrix, expressed in CO_2 equivalent (CO_2eq) (error bars are ± 1 SD, $n = 12$ at the first evaluation and $n = 6$ at the second and third evaluations). The treatments are represented as: (T1) Soil, with N, no BC; (T2) Soil, with N, BC 10 Mg ha^{-1} ; (T3) Soil, with N, BC 20 Mg ha^{-1} ; and (T4) Soil, with N, BC 50 Mg ha^{-1} . The control treatment (Soil, no N, no BC) is presented as “C”. Panels 1, 2 and 3 represent each gas sampling event after N application to soil, corresponding to 1st split N application at planting (1), 2nd split N application (2) and 3rd split N application (3)

determines the relative availability of NH_4^+ and NO_3^- for plant uptake but also the extent of losses via denitrification and leaching/runoff (JAT et al., 2014).

Table 4.3 presents the yield components of wheat obtained for the different treatments evaluated. Grain yield and 100-grain weight (*grain100*) increased as a function of BC application rate. T4 presented grain yield 58% and 27% higher than control and T1, respectively, and 100-grain weight was 24% (T2) to 36% (T4) higher relative to T1 ($P < 0.05$). Jeffery et al. (2011) estimated that 50 Mg ha^{-1} of BC would lead to a mean increase in crop productivity of approximately 28%, and a more recent meta-analysis looking at the range $41 - 50\text{ Mg ha}^{-1}$ of BC showed that the expected increase would be closer to 18% (JEFFERY et al., 2015).

Increased grain and total biomass yields was also observed with the application of BC + mineral N fertilizer to highly degraded sites, yielding 2.2 Mg ha^{-1} and 2.9 Mg ha^{-1} more than control plots (N fertilizer only) in the first and second year of maize cultivation, respectively (KIMETU et al., 2008). T1 presented shoot biomass 8% higher compared with the control, and the application rates of BC increased shoot biomass from 8% (T2) to 16% (T4) relative to T1 ($P < 0.05$) (Table 4.3). T4 also presented higher tillering compared with the control ($P < 0.05$), and there were no differences between T1, T2 and T3 ($P > 0.05$) (Table 4.3).

Plant height increased by a factor of 1.1 in T2 relative to T1, which in turn presented the lowest plant height among treatments ($P < 0.05$). Finally, no differences were observed regarding root biomass and number of head and spikelets among the evaluated treatments ($P > 0.05$).

Table 4.2 – Soil chemical attributes after 35 (before first N split application) and 120 days (harvest) of greenhouse pot experiment with and without ^{15}N -labelled fertilizer and application rates of BC

Properties	C	T1	T2	T3	T4	C	T1	T2	T3	T4
35 days						120 days				
pH	5.5±0.1 Bc	5.7±0.1 Ab	5.6±0.0 Abc	5.6±0.0 Abc	6.0±0.1 Aa	5.7±0.1 Aab	5.6±0.1 Ab	5.6±0.1 Ab	5.7±0.1 Aab	5.9±0.2 Aa
P	23.7±4.0 Ab	25.3±1.5 Ab	25.7±1.2 Ab	27.7±3.8 Aab	33.0±2.0 Aa	18.0±1.0 Ba	18.7±1.2 Ba	21.0±4.4 Aa	20.7±1.5 Ba	24.3±4.2 Ba
S	39.7±5.0 Aa	37.3±4.7 Aa	40.0±14.8 Ba	53.7±5.7 Aa	73.0±5.0 Aa	10.3±27.6 Bb	30.7±17.0 Bab	56.0±22.6 Aa	37.0±26.2 Bab	35.3±31.0 Bab
K	0.6±0.1 Ad	0.6±0.1 Ad	1.7±0.3 Ac	2.6±0.5 Ab	5.1±0.5 Aa	0.5±0.1 Ab	0.7±0.1 Ab	0.7±0.1 Bb	1.2±0.1 Bb	3.5±0.6 Ba
Ca	19.0±1.0 Aa	18.7±1.4 Aa	19.7±1.5 Aa	20.0±3.6 Aa	23.0±2.0 Aa	15.7±4.5 Aa	16.3±3.8 Ba	17.3±2.5 Aa	20.7±2.5 Aa	21.7±4.0 Aa
Mg	5.0±1.0 Aa	5.3±0.6 Aa	5.3±0.6 Aa	5.7±0.7 Aa	5.7±0.6 Aa	3.3±0.6 Aa	3.3±0.6 Aa	3.7±1.2 Aa	4.7±0.6 Aa	5.3±1.5 Aa
Ac	21.3±1.1 Aa	20.7±1.1 Aab	19.3±1.1 Aab	18.7±1.1 Ab	15.3±0.6 Ac	15.3±0.6 Bab	17.3±1.2 Ba	17.3±1.2 Ba	15.3±0.6 Bab	13.0±0.0 Bb
SB	24.6±2.0 Aa	24.6±2.0 Aa	26.7±2.0 Aa	28.3±4.2 Aa	33.8±2.2 Aa	19.5±5.1 Aa	20.3±4.2 Ba	21.7±3.7 Aa	26.5±2.6 Aa	30.5±6.2 Aa
CEC	45.9±2.3 Aa	51.9±3.1 Aa	46.0±2.2 Aa	48.9±3.2 Aa	49.2±2.6 Aa	34.8±5.6 Ba	37.7±3.5 Ba	39.0±2.6 Aa	41.9±2.6 Aa	43.5±6.2 Aa
BS	53.5±2.6 Ab	58.7±1.0 Aab	57.9±2.6 Aab	57.7±5.4 Aab	68.8±1.0 Aa	55.3±5.8 Ab	53.6±6.7 Ab	55.3±5.7 Ab	63.3±2.5 Aab	69.7±4.1 Aa
$NH_4^+ - N$	16.7±4.1 Aa	14.3±6.2 Ba	12.9±3.0 Ba	13.2±8.9 Aa	15.2±9.6 Aa	5.7±4.3 Ab	60.9±8.4 Aa	54.7±28.3 Aa	16.5±9.0 Ab	3.7±1.5 Ab
$NO_3^- - N$	1.0±1.7 Aa	< 0.01	< 0.01	2.7±5.1 Aa	2.4±3.1 Ba	5.0±1.0 Ab	< 0.01	4.6±1.8 Ab	2.9±6.3 Ab	17.3±4.1 Aa
Min. N	17.7±5.6 Aa	14.3±7.8 Ba	12.9±1.2 Ba	15.9±11.6 Aa	17.6±8.9 Aa	10.7±3.3 Ab	60.9±2.7 Aa	59.3±30.1 Aa	19.4±11.7 Ab	21.0±5.6 Ab

Mean ± standard deviation ($n = 6$, for 30 and $n = 6$, for 120 days). Means followed by the same uppercase letter compare within treatment between evaluation periods, and do not differ statistically at 5% probability by Tukey's test. Means followed by the same lowercase letter compare between treatments within evaluation period, and do not differ statistically at 5% probability by Tukey's test. The treatments are represented as: (T1) Soil, with N , no biochar; (T2) Soil, with N , biochar 10 Mg ha^{-1} ; (T3) Soil, with N , biochar 20 Mg ha^{-1} ; and (T4) Soil, with N , biochar 50 Mg ha^{-1} . The control treatment (Soil, no N , no biochar) is presented as "C". Ac: Potencial acidity; SB : Sum of bases; CEC : Cation Exchange capacity; BS : Base saturation; and Min N : mineral N .

Different crops have variable responses when grown with a BC soil amendment. Also, specific soil-BC interactions have yielded mixed results regarding crop yields. In general, important commercial crops such as rice, wheat, maize and soybean have shown significant increase in crop productivity of approximately 16, 17, 19 and 22%, respectively, when BC was added (JEFFERY et al., 2015), along with soils with a coarse or medium texture. Additionally, the combination of BC and mineral fertilizer have shown synergistic effects on plant growth compared to mineral fertilizer alone or pure BC (SCHULZ; GLASER, 2011).

One of the main mechanisms behind the reported positive effects of BC on crop yields may be its high pH and potential liming effect (JEFFERY et al., 2011). In this sense, the benefits following BC application to soil include the optimization of nutrient availability and use (e.g. mainly P , but also N , Ca and Mg) and the reduction of some elements toxic to plant growth such as Al^{3+} and Mn^{2+} . (STEINER et al., 2007) observed a large grain yield enhancement in rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L.) where charcoal with NPK fertilizer were applied to an acidic, highly weathered tropical soil. Also, a recent meta-analysis revealed that the greatest positive changes in crop productivity with BC addition was seen in the most acidic soils (pH ranging from 4 to 5.5) (JEFFERY et al., 2015).

The chemical composition of the BC also have shown positive effects on plant growth, since its ash fraction add free bases such as K^+ , Ca^{2+} , and Mg^{2+} to the soil solution, providing readily available nutrients for plant growth. Lehmann et al. (2003) concluded that the increase of rice and cowpea (*Vigna unguiculata* L.) biomass was attributed to K , P and, possibly, Cu provided by the BC itself. Despite the fact that BC themselves can have fertilization effects over short time scales, the input of bio-available nutrients can enhance plant total biomass and improve tissue quality, therefore influencing the quantity and quality of plant residues returned to the soil (KIMETU et al., 2008; JEFFERY et al., 2011; JONES et al., 2012).

Root-soil contact and water movement in the soil are also important for the delivery of mobile nutrients to growing crops (MARSCHNER, 2012) and thus improve plant growth and development. Therefore, a secondary effect of BC can be changes in plant nutrition by improving soil physical properties, which allows root growth and nutrient and/or water supply and uptake. Although there were no changes in root dry weight (Table 4.3), we speculate that the higher grain yields and nutritional quality with BC application may be partly attributed to differences in root distribution. Bruun et al. (2014) amended a hard-setting subsoil with 0.52% BC and found that wheat roots penetrated deeper and showed more branching and soil-volume exploitation in search for water and nutrients, which significantly improved wheat straw and grain yields.

Again, it should be highlighted that these effects are naturally far more pronounced in sandy than clayey soils (ABEL et al., 2013; CORNELISSEN et al., 2013; KINNEY et al., 2012), and with BCs that have relatively lower bulk densities, e.g., from wheat straw or *Miscanthus* (UTOMO, 2013.; BRECHT, 2013; KAMMANN; GRABER, 2015), maize plants (ABEL et al., 2013) or maize crop residues (KINNEY et al., 2012). Taken together, it is clear that BC

has several possible ways in which it may affect soil water supply and nutrient retention and delivery to plants, thus bringing positive effects on plant growth and grain yields. However, it should also be understood that none of these mechanisms works in isolation and there may be synergies or trade-offs between them or with other characteristics of BC that need to be more systematically investigated.

Table 4.3 – Wheat yield components of the treatments evaluated in the greenhouse pot experiment with and without ^{15}N -labelled fertilized and rates of BC application

Treatments	grain weight	100-grain weight	shoot biomass	root biomass	plant height	tiller	head	spikelet
	(g)		(g)		(cm)	(#)		
C	10.9 ± 3.0	2.4 ± 0.2	37.7 ± 3.6	5.3 ± 2.8	64.1 ± 3.5	14.7 ± 2.2	13.0 ± 2.5	17.4 ± 2.5
	C	B	C	A	AB	B	B	A
T1	13.5 ± 1.2	2.5 ± 0.2	40.7 ± 2.2	4.5 ± 0.5	62.0 ± 4.6	16.0 ± 2.5	15.4 ± 1.5	19.2 ± 2.0
	BC	B	C	A	B	AB	AB	A
T2	14.4 ± 1.9	3.1 ± 0.3	44.0 ± 2.0	6.0 ± 1.1	70.0 ± 5.2	16.3 ± 1.7	13.5 ± 1.6	18.2 ± 1.6
	AB	A	B	A	A	AB	AB	A
T3	15.9 ± 1.3	3.4 ± 0.4	45.9 ± 2.5	7.0 ± 1.7	67.0 ± 3.1	17.5 ± 1.5	13.7 ± 1.9	19.1 ± 2.7
	AB	A	AB	A	AB	AB	AB	A
T4	17.2 ± 0.7	3.2 ± 0.5	47.4 ± 2.8	6.3 ± 2.2	68.0 ± 2.6	19.3 ± 3.1	16.0 ± 2.6	18.4 ± 2.4
	A	A	A	A	AB	A	A	A

Mean ± standard deviation ($n = 6$). Means followed by the same letter in the columns do not differ statistically at 5% probability by Tukey's test. The treatments are represented as: (T1) Soil, with N, no BC; (T2) Soil, with N, BC 10 Mg ha^{-1} ; (T3) Soil, with N, BC 20 Mg ha^{-1} ; and (T4) Soil, with N, BC 50 Mg ha^{-1} . The control treatment (Soil, no N, no BC) is presented as "C".

Table 4.4 presents the nitrogen derived from the fertilizer ($Ndff$) and recovery ($\%R$) of the ^{15}N applied to the soil-plant system with and without BC amendment. No differences were observed in $Ndff$ and $\%R$ of roots and shoots biomass between treatments ($P > 0.05$). However, the treatments with N fertilizer + BC application presented significantly higher $Ndff$ in grains compared with no BC application. T3 and T4 presented $Ndff$ in grains 29% and 27% higher compared with T1 ($P < 0.05$), which correspond to 26% and 25% of the N-labelled fertilizer applied to soil, respectively, and to 20% in T1 (Table 4.4).

T2 and T3 presented the highest $Ndff$ and $\%R$ in the entire plant (roots + shoots + grains) compared with the other treatments, and the treatments with N fertilizer + BC application presented $Ndff$ and $\%R$ in soil 31% to 48% lower compared with T1 ($P < 0.05$) (Table 4.4). However, no differences were observed in $Ndff$ and $\%R$ of the soil-plant system between treatments ($P > 0.05$). Only a small fraction of the $Ndff$ was found in the BC within the pots in T2 to T4, ranging from 0.2% to 0.6% of the N dose applied to soil ($P > 0.05$). Finally, there were no differences among treatments regarding the loss of ^{15}N via gaseous emissions ($P > 0.05$) (Table 4.4).

Table 4.5 shows the correlation coefficients between the $\%R$ of the entire plant, $\%R$ of the soil, ^{15}N loss, biomass production and some soil properties. Considering all treatments evaluated, the correlation analysis showed that the recovery of ^{15}N by both plant and soil had a

significant positive and negative relationship, respectively, with the wheat biomass. Additionally, the remaining ^{15}N in soil related negatively with soil $p\text{H}$. In BC-amended soils the recovery of ^{15}N by the plants had a significant positive relationship with wheat biomass and related negatively with available P , while the remaining ^{15}N in soil was positively correlated to available P . Finally, the ^{15}N loss negatively related to wheat biomass.

Whether the biomass increases or not depends on nutrient availability (Table 4.5). Thus, these results clearly demonstrate increased N availability and N use efficiency by the wheat plants with BC addition to soil. Greater N use efficiency due to BC amendments had been reported in other studies (CHAN et al., 2007; ZWIETEN et al., 2010; JEFFERY et al., 2011; JONES et al., 2012) with several potential agronomic and environmental benefits.

Primarily, N availability and uptake has a major influence on crop's aboveground biomass. The way that N controls aboveground biomass expansion depends upon the stage of crop development. Before stem extension, N uptake affect aboveground biomass size by promoting tillering (SYLVESTER-BRADLEY et al., 2008), which was increased in the treatment with the highest BC dose in this study (Table 4.3). The main source of N during grain filling does not come from root uptake, but from a massive redistribution of N from the leaves and stems to form grain protein (SYLVESTER-BRADLEY et al., 2008). Considering a "aboveground biomass management" approach to N nutrition, it can be concluded that the combination of mineral N fertilizer and BC helped to achieve greater canopy size and adequate canopy survival through grain filling.

Retention of applied fertilizer N in soil can be increased by recycling of N taken up by the crop through inputs of plant residues (KIMETU et al., 2008; MAJOR et al., 2010). When urine-treated BC particles were applied to soil, Taghizadeh-Tossi et al. (2012) observed that subsequent plant growth was not affected but ^{15}N uptake into plant tissues increased, indicating that the adsorbed N was plant-available. Similarly, Kimetu et al. (2008) reported that N concentration in the plant tissue was about 2 g kg^{-1} above the levels of the control treatment (only mineral N fertilizer) with the addition of BC combined with mineral N fertilizer, respectively. In this sense, the lower ^{15}N remaining in soil with BC addition contrasts with the above findings, and it is mainly attributed to the pot trial conditions. On a highly weathered central Amazonian Ferralsol, Steiner et al. (2008) observed that N retention in soil was significantly higher from first to second harvest in the charcoal-amended plots (16% of the N applied) in comparison to only mineral-fertilized plots (10% of the N applied). The enhanced ^{15}N recovery in soil was due to remaining crop residues and increased retrieval of N rather than higher total soil N contents.

Zheng et al. (2013) found a lower ratio of total N accumulated to total root length and a higher ratio of produced biomass to unit of N uptake, suggesting that less N was absorbed by maize but more biomass was produced using the equivalent amount of N in the BC-amended soil compared to the control, and attributed this result to the lock up of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$ onto BC structure when applied to soil. Additionally, Güereña et al. (2013) have provided evidence of

improved N use efficiency through transient incorporation of N into microbial biomass, which may be a result of mineralization of a comparatively labile fraction of BC with a high C/N ratio (LEHMANN et al., 2003; RONDON et al., 2007; LAIRD et al., 2010).

However, the greater N concentrations in the grain of the wheat plants that received BC did not suggest N immobilization, but rather a net increase in N availability, which related well to the high recalcitrance of BC against microbial attack (See Chapter 2). Decomposition of BC was probably minimal and therefore significant N immobilization may not have occurred. Other studies have reported increased N uptake and N use efficiency due to the effects of BC on soil fertility and structure. Chan et al. (2007, 2008) found enhanced N use efficiency under pot trial with application of poultry and green biomass BCs on radishes (*Raphanus sativa* L.), and attributed this to increases in soil pH and available P , along with improved soil structure.

The results obtained for ^{15}N loss and N_2O fluxes provide some indication of gaseous N losses through enhanced complete denitrification (i.e. N_2 as a final product) with BC application to soil, since there were no differences among treatments in ^{15}N gaseous loss and BC application resulted in lower N_2O fluxes. This hypothesis have been previously investigated and mainly attributed to two factors: (i) the generation of a redox potential within BC's pores (OH; SON; CHIU, 2013); and (ii) the acid neutralization capacity of BC, which together may promote a shift of the product ratio $N_2O : N_2$ towards N_2 (DORSCH; BRAKER; BAKKEN, 2012). As discussed in Chapter 3, BC can act as an "electron shuttle", facilitating the transfer of electrons to soil denitrifying microorganisms, which together with its liming effect would accelerate the last step of denitrification (CAYUELA et al., 2013).

The N cycle is intrinsically linked to soil water conditions, so if BC incorporated into soil creates an uneven distribution of water between the soil and BC phases, then a boundary will develop (CLOUGH et al., 2010). O'Neill et al. (2009) found differences in bacterial community composition when comparing Brazilian Anthrosols and adjacent soils. Thus, this soil-BC boundary could potentially drive changes in soil microbial composition and function with respect to N cycling. Additionally, increasing pH levels clearly decrease N_2O emissions by alleviating the inhibition of N_2O reductase in acid soils (LIU et al., 2010).

The results of this chapter show that BC application to soil can potentially enhance N bioavailability and N use efficiency, while simultaneously decreasing emissions of N_2O (ZWIETEN et al., 2010). In this sense, the implications of BC soil management systems are mainly related to reduced cultivation costs and N_2O emissions derived from the application of mineral N fertilizers to soil, besides their impacts on soil quality and hence crop yields.

Table 4.4 – Nitrogen derived from the fertilizer (*Ndf f*) and recovery percentage of the $NH_4[^{15}N]O_3$ applied to the soil-plant system with and without ^{15}N -labelled fertilizer and application rates of BC

	Nitrogen derived from the ^{15}N -fertilizer (<i>Ndf f</i>) (mg per pot)				Recovery of the <i>N</i> fertilizer (%)			
	<i>T1</i>	<i>T2</i>	<i>T3</i>	<i>T4</i>	<i>T1</i>	<i>T2</i>	<i>T3</i>	<i>T4</i>
roots	5.5±0.7 A	7.2±1.9 A	8.2±2.3 A	7.5±3.4 A	1.5±0.2 A	1.9±0.5 A	2.2±0.6 A	2.0±0.9 A
shoots	73.5±12.6 A	79.2±15.4 A	77.4±10.8 A	74.4±13.0 A	19.5±3.3 A	21.0±4.1 A	20.5±2.9 A	19.7 ±3.4 A
grains	75.4±13.7 B	92.2±12.2 AB	97.5±11.4 A	95.7±18.1 A	20.0±3.6 B	24.4±3.2 AB	25.8±3.0 A	25.4±4.8 A
entire plant	154.4±24.6 B	178.6±20.7 A	183.1±18.0 A	177.6±33.8 AB	40.9±6.5 B	47.3±5.5 A	48.6±4.8 A	46.6±8.9 AB
soil	86.9±52.0 A	59.9±37.8 B	44.9±29.3 B	47.6±33.1 B	23.0±13.8 A	15.9±10.0 B	11.9±7.8 B	12.6±8.8 B
soil-plant	241.3±42.1 A	238.5±24.5 A	228.0±24.9 A	225.2±6.2 A	64.0±11.2 A	63.2±6.5 A	58.5±6.6 A	61.0±1.6 A
BC	.. A	2.2±3.0 A	0.8±1.2 A	2.1±2.1 A	.. A	0.6±0.8 A	0.2±0.3 A	0.6±0.6 AB
^{15}N loss	135.7±42.1 A	136.2±27.3 A	155.7±25.7 A	142.0±3.5 A	36.0±11.2 A	36.1±7.2 A	39.1±4.6 A	37.7±0.9 A

Mean ± standard deviation ($n = 6$). Means followed by the same letter do not differ statistically at 5% probability by Tukey's test. The treatments are represented as: (*T1*) Soil, with *N*, no BC; (*T2*) Soil, with *N*, BC 10 $Mg\ ha^{-1}$; (*T3*) Soil, with *N*, BC 20 $Mg\ ha^{-1}$; and (*T4*) Soil, with *N*, BC 50 $Mg\ ha^{-1}$ . ..: Not available.

Table 4.5 – Correlation coefficients between the %R in entire plant, %R in soil, ^{15}N loss, biomass production and some soil properties (n = 6)

Treatments	Properties	<i>pH</i>	<i>P</i>	<i>K</i>	above biomass	below biomass	total biomass
<i>T1 – T4</i>	%R plant	0.39	-0.25	0.06	0.58**	0.46*	0.59**
	%R soil	-0.47**	0.19	-0.17	-0.70**	-0.43*	-0.68**
	^{15}N loss	0.23	-0.24	0.01	0.48*	0.18	0.45
w/o BC [†]	%R plant	0.21	0.04	0.58	0.37	-0.62	0.31
	%R soil	-0.26	0.42	0.68	-0.74	0.82	-0.72
	^{15}N loss	0.20	-0.54	-0.64	-0.62	0.71	-0.64
w/BC [‡]	%R plant	0.31	-0.56*	-0.13	0.46*	0.42*	0.50*
	%R soil	-0.20	0.53*	0.12	-0.25	-0.44	-0.34
	^{15}N loss	-0.45	0.46	-0.04	-0.64**	-0.50*	-0.66**

[†]w/o BC includes *T1* (mineral *N* fertilizer only)

[‡]w/ BC includes *T2* to *T4* (mineral *N* fertilizer + BC)

* $P < 0.05$ and ** $P < 0.01$.

4.4 Conclusions

1. The application of sugarcane straw biochar to soil improved *N* availability and the efficiency with which *N* is acquired by the plant and converted to grain yield, thereby enhancing crop performance; and
2. The biochar amendment significantly reduced N_2O emissions that result from the application of nitrogen fertilizer to soil.

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5 FINAL CONSIDERATIONS

The BC produced from sugarcane straw present desirable characteristics as a soil amendment and provide soil carbon sequestration. Interesting aspects were observed regarding its characterization, reflecting the effects of pyrolysis on the chemical and physical transformation of the sugarcane straw into BC.

First, the pyrolysis conditions adopted in this study resulted in a stable final product that could attend different purposes when applied to soil. The morphological analysis showed that the BC keeps great structural similarity with the original biomass. In other words, the slow pyrolysis at 450°C did not promote plastic deformation and structural loss by melting and volatilization, and allowed to produce a BC with a wide range of pores with suitable dimensions for clusters of microorganisms to inhabit.

Moreover, its high pH and total contents of exchangeable cations indicated that this BC may have potential to mitigate some soil-health constraints to crop productivity, such as soil acidity, exchangeable Al^{3+} and percent Al saturation. Finally, the incubation study showed that its stable carbonaceous phase makes BC a potential carbon sequestration strategy.

Altogether, this study opens encouraging perspectives such as the evaluation of the sugarcane straw as a chemical feedstock and an alternative biofuel through pyrolysis, providing greater amounts of a desirable renewable that can improve soil quality besides acting as a climate change mitigation strategy.

The BC produced from sugarcane straw combined with organic fertilizers have synergistic effects on soil chemical attributes and on the mitigation of greenhouse gases emissions. The study revealed soil-specific BC interactions, since the soil with a comparatively lower baseline nutrient concentration was more responsive to BC application in terms of improvements in soil chemical attributes, and presented more synergistic effects from the combination of BC with organic residues.

Also, the results found in this study helped to confirm some of the findings from the BC characterization, mainly regarding its impacts on soil pH and availability of basic cations, and revealed other aspects such as a consequential increase in P availability, reinforcing the use of the produced BC as a potential option for maintaining and/or improving soil chemical properties, while simultaneously mitigating N_2O emissions from the residues commonly applied to sugarcane fields.

Additionally, N_2O emissions were reduced as a function of BC application rate. However, future research on this topic must be carried out close to natural conditions to offer a more realistic approach of this complex dynamic.

The BC produced from sugarcane straw mitigate nitrous oxide emissions and changes nitrogen availability to plants. It could be testified that BC addition to soil not only successfully reduced N_2O emissions derived from the application of mineral N fertilizer, but also improved plant N nutrition and crop performance.

The combination of mineral N fertilizer and BC changed the efficiency with which N is acquired by the wheat plants and redistributed to the grains, which increased the aboveground biomass and the retention of the applied N fertilizer in grains. The main implications for agriculture are related to reduced costs of cultivation, due to enhanced N bioavailability and N use efficiency, as well as the N recycling through inputs of plant residues, and reduced impacts of N fertilization on N_2O fluxes.

Although the ^{15}N of the N_2O emitted was not directly determined in this study, the results observed in this study provide some indication that the mechanism responsible for reducing gaseous N losses as N_2O could be enhanced complete denitrification with BC application to soil.

Further investigations on this topic will permit more accurate estimation of the mechanisms responsible for this reduction and the assessment of the actual duration of these impacts. This is fundamental to assess the real climate change mitigation potential from the application of sugarcane straw, and to establish the best management practices when incorporating BC to soil.

Additionally, the implications of BC application on environmental conditions need to be assessed across different environments, soil types or soil and crop combinations in order to expand our knowledge on this research in Brazil and worldwide.