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Effects of Biochar on the Emissions of Greenhouse Gases from Sugarcane Residues Applied to Soils

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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 may be significant sources of greenhouse gases emissions to the atmosphere. This study assessed the impact of sugarcane straw biochar on the emissions of CO_2, $CH_4$and N_2O promoted by filter cake and vinasse applied to soil, and its effects on the chemical properties and bacterial communities of a Typic Hapludox and a Quartzipsamment. A laboratory incubation was conducted for 100 days with both soils under five treatments: vinasse and filter cake amendment (FV), plus biochar at 10 (FV + B10), 20 (FV + B20) and 50 (FV + B50) $Mg \cdot ha^{-1}$, and a control. Soil pH, available P and exchangeable base contents increased with biochar added to sandy soil. Mineral N decreased with biochar addition to both soils. The FV treatment increased CO_2 emissions by 5-fold and 2.4-fold in sandy and clayey soils, respectively, compared to the control. Moreover, FV +B10 increased CO_2 emissions by 4% and 6.4% in sandy and clayey soils, respectively, compared to FV. Cumulative N_2O emissions in FV were 537% and 125% higher in sandy and clayey soils, respectively, compared to the control. Nevertheless, increasing biochar amendment rates reduced N_2O emissions from 24% to 34% in sandy soil, and from 14% to 56% in clayey soil. CH_4 emissions were negligible. The effects of filter, vinasse and biochar amendments on soil amelioration were closely related to its buffering capacity. Temporal changes on bacterial community structure were more pronounced in the sandy soil compared to clayey, and indicated that N_2O emission mitigation in clayey soil was

directly related to biotic mechanisms, while abiotic mechanisms caused by biochar played a more important role in mitigating N_2O emissions in sandy soil.

Keywords

Filter Cake, Fingerprinting, Nitrous Oxide, Pyrolysis Carbon, Soil Fertility, Vinasse

1. Introduction

The intensification of green cane harvesting has led to a greater deposition of leaves and tips on soil surface, ranging between 10 and 20 $Mg \cdot ha^{-1}$ of dry matter, and the amount of sugarcane crop residues generated in Brazil is estimated in 175 million $Mg \cdot yr^{-1}$ [1]. Against the claiming demand to use this biomass for bioenergy generation, the Brazilian sugarcane sector has considered the partial removal of the post-harvest residues from soil surface without harming sustainability and yields [2]. On the other hand, 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 [3].

Vinasse is an acidic (pH ≈ 4.5) nutrient-dense effluent that is produced at a rate of approximately 13 L for every liter of ethanol. Filter cake is a nutrient-rich solid residue from the filtration of sugarcane juice, produced in an average of 8 kg per ton of processed sugarcane [4]. Despite vinasse and filter cake benefits to conservation agriculture [3], these residues may be significant sources of greenhouse gases (GHG), mainly nitrous oxide (N_2O) and methane (CH_4) [5] [6] [7].

In this context, one of the proposed means to reduce GHG emissions in agriculture is through the use of biochar (charcoal derived from the pyrolysis of biomass). Despite the benefits of biochar applications to soil [8]-[16], studies regarding its combination with other organic residues are still limited. Positive interactions between biochar and organic residues can be expected due to the biological activation of biochar and reduced organic fertilizer mineralization, leading to synergisms between biochar and organic residues [17].

According to [18], the combination of biochar with poultry manure reduced N losses by volatilization and produced high quality composts. [17] showed that biochar addition to soil in combination with organic fertilizer can stabilize compost-derived organic matter (OM) and increase soil C sequestration, as well as improve soil fertility over the sole biochar or organic/mineral fertilizer application. Biochar-amended soils have also shown to reduce CO_2emissions [19] in response to vinasse application.

Under the current scenario of climate change, the combination of biochar with organic residues may be an approach to improve nutrient cycling and to fulfill non-agronomic purposes, such as reduction of GHG emissions. The aim of this study was to assess the effects of applying sugarcane straw biochar com-

bined with vinasse and filter cake on the emissions of CO_2, CH_4 and N_2O, chemical properties and bacterial community composition of two contrasting soils (*i.e.* clayey and sandy tropical soils). It was hypothesized that: i) The effects of biochar amendments on soil amelioration is closely related to soil buffering capacity; ii) biochar suppresses GHG emissions from filter cake and vinasse applied to soils as a function of its application rate; and iii) soil-biochar interactions cause temporal changes in bacterial communities both directly and indirectly, affecting niche-microbe interactions related to N_2O emission mitigation. For testing these hypotheses an incubation experiment was conducted under controlled environmental conditions (*i.e.*, temperature and moisture), with and without application of vinasse and filter cake combined with addition of biochar at different rates in two contrasting forest soils.

2. Materials and Methods

2.1. Biochar Production and Characterization

The feedstock for biochar production was straw collected from a sugarcane field within a mill located in Piracicaba, State of Sao Paulo, Brazil. A recently harvested area (*i.e.*, 7 days after unburned mechanized harvesting) was selected since it presented a largeamount of fresh post-harvest residues on soil surface (≈ 10 $Mg \cdot ha^{-1}$ of dry matter).

Before pyrolysis, the straw particles were cut into fragments of 5 ± 1 cm. Then, the reactor was cleaned under heating with air injection in order to remove impurities prior to allocation of the raw material. Approximately 3 kg of feedstock was manually placed into the sample port of the reactor, which consisted of 300- × 2400-cm steel cylinder (diameter × length) closed on one end with a circular steel plate.

The pyrolysis process was carried out under N_2atmosphere, with a final temperature of 450˚C (Δ ≈ 20˚C) and heating rate of $10˚C \cdot min^{-1}$ for a retention time of 2 hours. The condensable gases were recovered on the other end of the reactor as a liquid (*i.e.* bio-oil). Non-condensable gases were exhausted to a water tank outside the processing unit to avoid their direct release to the atmosphere.

After completion of pyrolysis, the sample presented homogeneous carbonization and a volume reduction of 30% to 40%. The pyrolysis process yielded 30% of biochar, 40% of liquids (bio-oil) and 30% of gas, which is within the range observed in most studies for slow pyrolysis [20]. Chemical properties of the feedstock and final biochar are presented in **Table 1**.

2.2. Soils and Organic Residues

Two soils with contrasting texture, a Quartzipsamment (sandy) and a Typic Hapludox (clayey), were collected from two different native forest areas located, respectively, from near Anhembi town, State of Sao Paulo, Brazil (22˚43'31.1"S; 48˚01'20.2"W) and within the ESALQ campus (22˚42'05.1"S; 47˚37'45.2"W), Piracicaba, respectively; both located at the State of Sao Paulo, Brazil. Native vege-

Table 1. Characterization of the feedstock (sugarcane straw) for biochar production, biochar, filter cake and vinasse used in the study.

Residues	C	N	C/N	P	Ca	Mg	K
	mg·g^{-1}			mg·g^{-1}			
Straw	5.8 (0.1)	479.0 (12.0)	8.0 (2.0)	59.9 (1.3)	1.0 (0.1)	7.7 (0.2)	1.5 (0.5)	9.5 (3.5)
Biochar	9.2 (0.6)	674.6 (35.4)	13.8 (1.5)	48.9 (4.2)	2.2 (0.9)	5.1 (1.1)	3.2 (0.7)	12.5 (2.2)
Filtercake	6.5 (1.1)	133.9 (27.2)	8.4 (2.3)	15.9 (8.9)	10.5 (1.1)	10.0 (0.8)	1.7 (0.3)	1.4 (0.5)
Vinasse	4.7 (0.8)	17.0 (8.3)	0.8 (0.2)	21.2 (4.4)	0.6 (0.2)	0.8 (0.2)	0.3 (0.1)	3.5 (0.8)

Mean (SD), n = 3.

tation (seasonal semideciduous forest) soils were chosen to avoid residual effects of filter cake and vinasse application on soil properties [21] [22] [23]. These soils were sampled at the 0 - 20 cm layer, air-dried, homogenized, and sieved to 2 mm before installing the experiment. Soil characteristics are given in **Table 2**.

Both the filter cake and vinasse were collected fresh from a sugarcane mill located in Piracicaba, State of Sao Paulo, Brazil. Prior the application to experimental units, filter cake was dried at 45˚C by 48 h in a forced-air oven, and the dried material was gently crushed and sieved (<2 mm) before incubation. Vinasse was kept frozen at −20˚C until use.

The pH and chemical characteristics differed considerably among materials (**Table 1**). Paired comparisons using a Tukey-Kramer HSD test showed that biochar was richer in nutrients ($p < 0.05$), while vinasse showed the lowest concentrations of nutrients among residues.

2.3. Experimental Set-Up and Design

The laboratory incubation was conducted with two soils (*i.e.* sandy and clayey) under five treatments: filter cake and vinasse amendment (FV), plus biochar at three application rates (FV + B10, FV + B20 and FV + B50), and control (soil-only). Biochar was applied at 0.4%, 0.8% and 1.9% (w/w) to sandy soil, and at 0.5%, 1% and 2.5% (w/w) to clayey soil. These additions represent field application rates of 10, 20 and 50 Mg·ha^{-1} of biochar to soil (assuming an incorporation depth of 20 cm and considering the bulk density of 1.0 and 1.3 g·cm^{-3} for clayey and sandy soil, **Table 2**). These were then placed in airtight glass jars of 1.4 L and pre-incubated at 4˚C for 24 h to minimize the disturbance effects on microbial communities and soil processes, before starting the incubation at 25˚C by 100 days.

The amount of filter cake and vinasse applied to all treatments was equivalent to 100 Mg·ha^{-1} and 100 m^3·ha^{-1}, respectively, which are the application rates commonly used in Brazilian sugarcane fields [24]. Biochar, filter cake and vinasse were thoroughly mixed with the dry soil to obtain a completely homogeneous mixture, and soil moisture was adjusted to 60% water-filled pore space (WFPS). Replicate jars from each treatment were destructively sampled at dif-

Table 2. Properties of two incubated soils (0 - 20 cm depth) used to evaluate the combination of biochar with organic residues from sugarcane industry.

Soils	Sand/Silt/Clay	Bulk density	pH	C	N
	%	$g \cdot cm^{-3}$		$mg \cdot g^{-1}$	
Quartzipsamment (sandy)	90/2.2/7.8	1.3 (0.2)	4.1 (0.1)	13.0 (1.0)	1.0 (0.0)
TypicHapludox (clayey)	40/28/32	1.0 (0.1)	6.2 (0.0)	39.0 (2.0)	3.0 (0.0)

ferent times (n = 2 at 30 days and n = 4 at 100 days) to characterize soil chemical attributes and bacterial communities.

2.4. Soil Chemical Characteristics

To assess the chemical characteristics of the incubated soils over time, destructive sampling was performed after 30 and 100 days of incubation, by removing four replicates per treatment at 30 days (n = 4), and the four remaining replicates after 100 days. Subsamples were kept at −20˚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 1 M KCl solution. Extracts were immediately frozen and kept for further measurement using the flow injection analysis method [25].

The pH was determined in H_2O using a biochar: solution ratio of 1:2 (w:v) and agitation at 220 rpm for 30 min. Samples were left to settle for 30 min before pH was determined with a pH electrode. Available P and exchangeable cations (K^+, Ca^{2+}, Mg^{2+}) were determined according to the method proposed by [26]. Sulfur content was determined by extraction in monocalcium phosphate 0.01 M and subsequent quantification by colorimetry [27]. The exchangeable acidity was determined by extraction of $H^+$and Al^{3+} with a $Ca(OAc)_2$1.0 M solution buffered to pH 7 [27].

2.5. Gas Sampling

The fluxes of CO_2, N_2O and CH_4 in each treatment were estimated by determining the concentration of gases in the jars' headspace over the experimental duration. Each incubation unit (*i.e.* glass jar containing each replicated treatment) was closed and samples of the headspace gas were taken at time zero and final using 20 mL syringes. After completion of sampling event (*i.e.* gas buildup), the jars were opened to flush out its gaseous contents and closed again for the next sampling, which occurred daily for the first 7 days of incubation and after this period was performed at intervals of 2 to 3 days until final incubation time (100 days).

The concentrations of CO_2, N_2O and CH_4 at each sampling time were determined using a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, USA), and daily fluxes of CO_2 (mg CO_2–$C \cdot m^{-2} \cdot day^{-1}$), N_2O (μg N_2O–$N \cdot m^{-2} \cdot day^{-1}$) and CH_4 (μg CH_4–$C \cdot m^{-2} \cdot day^{-1}$) were calculated from the time versus gas concentration data using linear regression. These data were then used to calculate the cumulative emissions by the linear interpolation of data points between days and numerical integration of the area under curve using the trapezoid rule [28].

2.6. Bacterial Communities

After 30 and 100 days of incubation, samples of 400 mg of soil from each treatment were subjected to a total DNA extraction using the Power Soil DNA isolation kit (Mo Bio, Carlsbad, EUA), following the manufactory instructions. DNA extraction and integrity were assessed by 1% agarose gel electrophoresis performed at 100 W and 400 mA for 50 min, followed by staining with ethidium bromide and photo documentation under ultra-violet light (transluminator, Storm 845-GE Healthcare Life Sciences, Piscataway, NJ, USA).

The amplification of the V6 region of ribosomal gene 16S rDNA was performed with primers F968/GC [29] and R1378 [30]. The PCR reaction was performed in a total volume of 50 μL, with each reagent in final concentration of 1X PCR Buffer; 0.2 mM of each dNTP; 3.5 mM of MgCl2; 0.2 pMol of each oligonucleotide; 1U-Taq DNA polymerase (Fermentas, Burlington, Canada); and 1 μL of DNA sample (20 ng).

The PCR reaction was run in Veriti Thermal Cycler (Applied Biosystems, Waltham, USA) in the following reaction conditions: initial denaturation at 94˚C for 3 min, followed by 35 cycles of denaturation at 94˚C for 1 min, 53˚C for 40 s, extension at 72˚C for 40 s and a final extension at 72˚C for 10 min.

The DGGE was performed using the phorU2 systems (Ingeny International, Goes, The Netherlands). The PCR products were loaded onto 6% (w/v) polyacrylamide gels with denaturing gradients of 45% - 65% (urea 7 M and formamide 40%). The gels were run for 16 h at 100 V and 60˚C and stained with SYBR Green I (Invitrogen, Breda, The Netherlands). The DGGE gels were photodocumented with Storm 845 (General Electric) and analyzed using the Image Quant TL unidimensional (Amersham Biosciences, Amersham, UK, v.2003) [31], where band patterns were converted into abundance matrices of bands.

2.7. Statistical Analyses

Model residuals were tested for normal distribution using quantile-quantile plot and the Shapiro-Wilk test. A nested analysis of variance (Nested ANOVA) was carried out to the results regarding soil chemical attributes. Post-hoc Tukey HSD test was applied for the comparison of mean values between and within treatments (over the incubation period). The mean cumulative GHG fluxes obtained for all treatments were also submitted to ANOVA and Tukey test. All statistical analyses were carried out using the software R [32]. Regarding the bacterial groups, a Permanova test was performed to describe the significance of incubation period, doses of biochar and their interaction under 999 random permutations. Within these parameters, it was perfomed a principal coordinate analysis (PCoA) based in the BrayCurtis algorithm [33]. In addition, analysis of similarities (ANOSIM) was conducted to determined the significance of grouping patterns. These statistical analyses were performed using Past Statistics 1.90 program [34].

3. Results and Discussion

3.1. Sugarcane Residues and Biochar Effects on Soil Chemical Attributes

Biochar amendment at 50 Mg·ha^{-1} (FV + B50) to sandy soil significantly increased pH after 30 days of incubation compared to the other treatments (Table 3). However, the subsequent evaluation (100 days) showed a significant decrease in soil pH among treatments (Table 3). In contrast, application of FV plus biochar did not have a significant effect on pH in clayey soil (Table 3).

Table 3. Chemical attributes of sandy and clayey soil matrixes after 30 and 100 days of incubation with sugarcane straw biochar combined with residues from the sugarcane industry.

Treatments	pH	P	Ca	Mg	K	H + Al	NH_4^+	NO_3^-
		mg·dm^{-3}		mmol$_c$·dm^{-3}			mg·kg^{-1}	
				Sandy-30 days				
Control	4.3 Ac	8.0 Ac	3.3 Ac	3.3 Ac	1.6 Ad	15.6 Aa	17.3 Ab	51.9 Ab
FV	6.3 Ab	53.6 Ab	22.7 Aab	7.0 Ab	3.8 Ac	5.3 Ab	24.7 Aa	62.5 Aa
FV + B10	6.2 Ab	68.3 Ab	25.7 Aa	8.0 Aab	8.9 Bb	5.1 Ab	7.0 Abc	22.1 Bc
FV + B20	6.2 Ab	67.0 Ab	26.3 Aa	8.0 Aab	12.0 Bb	5.1 Ab	9.2 Ab	27.9 Bc
FV + B50	6.6 Aa	92.0 Aa	20.7 Ab	9.0 Aa	14.5 Ba	4.8 Ab	6.1 Ac	30.9 Ac
				Sandy-100 days				
Control	4.1 Ad	7.0 Ac	3.7 Ac	3.0 Ab	1.3 Ad	16.0 Ba	1.2 Cc	56.9 Aab
FV	5.2 Bc	28.0 Bb	15.0 Bb	6.7 Aa	3.9 Ac	6.0 Ab	1.0 Cc	49.5 ABb
FV + B10	5.4 Bbc	40.7 Bab	21.0 Ba	8.0 Aa	10.3 Ab	5.8 Ab	1.9 Bbc	68.1 Aa
FV + B20	5.6 Bab	35.7 Bb	20.3 Ba	7.7 Aa	13.6 Ab	5.7 Ab	4.1 Ba	61.1 Aa
FV + B50	5.8 Ba	55.3 Ba	21.0 Aa	8.0 Aa	15.5 Aa	5.8 Ab	3.2 Bab	36.6 Ab
				Clayey-30 days				
Control	6.5 Aab	28.0 Ac	96.5 Bb	20.0 Bc	4.0 Ac	12.0 Ba	36.5 Aa	89.2 Aa
FV	6.7 Aa	98.5 Aab	103.5 Bb	23.6 Cb	9.1 Aab	5.0 Bbc	46.9 Aa	125.7 Aa
FV + B10	6.6 Aab	116.0 ABab	142.0 Ba	29.6 Aa	7.0 Abc	6.0 Bb	12.8 Ab	38.4 Bb
FV + B20	6.5 Aab	131.5 Aa	105.0 Cb	28.0 Ba	8.6 ABab	6.0 Bb	10.5 ABb	46.8 Bb
FV + B50	6.5 Ab	70.5 Abc	97.0 Bb	26.0 ABab	10.7 Aa	5.0 Bb	10.9 Ab	42.5 Bb
				Clayey-100 days				
Control	5.8 Bb	20.0 Ab	166.0 Aa	27.0 Ac	3.5 Ac	12.5 Aa	1.6 Ba	79.5 Aa
FV	6.3 Ba	87.0 Aa	164.0 Aa	36.0 Aa	5.2 Bbc	6.0 Ab	2.1 Ba	64.5 Ba
FV + B10	6.3 Ba	120.0 Aa	165.0 Aa	33.0 Aa	5.7 Ab	5.0 Abc	4.6 Aa	71.3 Aa
FV + B20	6.3 Ba	81.5 Ba	160.5 Aab	32.0 Aab	6.1 Bab	5.3 Ac	2.0 Ba	85.8 Aa
FV + B50	6.3Ba	102.0 Aa	146.0 Ab	28.0 Abc	6.9 Ba	5.3 Bd	2.8 Aa	58.3 Ba

Values presented are means, n = 4 for 30 and 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: soil with filter cake and vinasse (FV); plus biochar at 10 (FV + B10), 20 (FV + B20) and 50 (FV + B50) Mg·ha^{-1}.

The application of FV + B50 in sandy soil increased available P by 72% higher compared to FV after 30 days of incubation (**Table 3**). However, available P concentrations over time behaved similarly to pH, with a decrease varying between 40 (FV + B50) and 48% (FV) after 100 days of incubation.

Increased soil pH have been attributed to biochar richness in alkaline cations suchas Ca^{2+}, Mg^{2+} and K^{+}, which are released through dissolution of the mineral phase to the soil solution [8] [15].

The further reduction in soil pH observed over time may be an effect of vinasse (**Table 3**). [35] observed increase of soil pH as function of vinasse dose applied to soil. However, after 100 days of incubation the soil pH where vinasse was applied reached that of the control, indicating its transient effect on pH levels. It is important to note that the steeper decrease of pH in vinasse and filter cake amendent compared to increasing additional biochar application (**Table 3**) can be assigned to the real potential of pure biochar to improve soil acidic conditions.

The increase in sandy soil pH caused by biochar precipitated ions $Al^{3+}$and decreased potential acidity (**Table 3**), which often are major constraints for agricultural productivity in highly weathered tropical soils [36]. This pH rise also led to a greater P availability from filter cake, a rich-P residue (**Table 1**) with about 50% of its total P available in the short-term under favourable soil conditions [24]. As expected, the non-significant changes in pH for the clayey soil (**Table 3**) indicate that the extent of these changes will strongly depend on the soil pH-buffering capacity [14].

All applications increased the cation exchange capacity (CEC) in sandy soil (**Table 3**). After 100 days of incubation, CEC increased from 43% in the FV + B10 to 59% in the FV + B50 treatment compared to FV ($P < 0.05$). In contrast, applications did not have a significant effect on CEC of the clayey soil after 100 days ($P > 0.05$).

The impacts of organic amendments on cation exchange capacity (CEC) are generally more pronounced in sandy soils, while for soils that already contain higher levels of organic matter and clay these impacts may be inconsequential [13]. The results show that nutrient retention can be improved even more by the addition of biochar to soils, especially to those with low ion-retention capacity. It is assumed that slow oxidation occurs on the edges of the aromatic backbone of biochar by both biotic [37] and abiotic processes [10], forming carboxylic groups and sustainably increasing CEC [38].

After 30 days of incubation, the FV treatment increased the concentrations of NH_4^+-N and NO_3^--N in sandy soil by 43% and by 20%, respectively, compared to the control (**Table 3**). In contrast, in this same period, increasing doses of biochar resulted in a decreased of the mineral N concentrations (by 63% in the case of FV + B10 and by 75% in the case of FV + B50 for NH_4^+-N; and by 50% (FV + B10) and 65% (FV + B50) for NO_3^--N) compared to FV ($P < 0.05$). After 100 days of incubation, it was observed a steep decrease in NH_4^+-N followed by a concomitant increase in NO_3^--N among treatments in sandy soil.

The FV treatment in clayey soil did not affect NH_4^+-N and NO_3^--N concentrations compared to the control ($P > 0.05$) (**Table 3**). Biochar addition decreased the mineral N concentrations in clayey soil compared to FV by 76 for NH_4^+-N and 66% for NO_3^--N after 30 days ($P < 0.05$). There were no significant differences as a function of biochar application rates ($P > 0.05$). Nevertheless, the subsequent evaluation (100 days) showed no significant differences between applications and the control for both NH_4^+-N and NO_3^--N concentrations ($P > 0.05$).

The initially lower mineral N with increasing biochar rate to both sandy and clayey soils (**Table 3**) may indicate the adsorption of NH_4^+-N and NO_3^--N onto biochar. Indeed, biochar may tighten the soil N cycling through direct sorption of mineral N, organic N compounds, enzymes and gases, including N_2O [11] [39] [40]. It has been suggested that physical entrapment in biochar pores may be responsible for removing NH_4^+ from soil solution, which is a possible mechanism given the diameter of the NH_4^+ ion (286 pm) and the wide range of pore sizes in biochars [41].

The enhanced NO_3^- concentrations in soil solution at biochar amendments of 10 and 20 $Mg \cdot ha^{-1}$ after 100 days of incubation, especially in sandy soil (**Table 3**), may be due to the lower ability of biochar to retain NO_3^-compared to NH_4^+ [39] [42]. Biochar can sorb NH_4^+ through acid surface functional groups (e.g. carboxyl and hydroxyl) via cation exchange [9], thus reducing its availability for autotrophic conversion to NO_3^- for some period of time.

Also, autotrophic nitrification may have been stimulated by high pH microsites of biochar [11]. Recently, [43] have found that nitrification was increased due to a greater NH_4^+ substrate supply for autotrophic nitrifiers. As the exact mechanism involved in the adsorption of N forms onto biochar and the effect of time on these processes remain to be understood, all of these phenomena are possible explanation for the enhanced nitrification with biochar addition to soil.

The results of mineral N also suggest that some microbial N immobilization had also been taking place at the highest rate of biochar amendment, since the removal of NH_4^+ and NO_3^- from soil solution prevailed after 100 days of incubation for this treatment (**Table 3**). In this case, the highest biochar 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.

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 biochar and its combination with organic residues on soil chemical attributes.

3.2. Cumulative GHG Emissions from Sugarcane Residues and Biochar

The FV application increased CO_2 emissions from the sandy soil by 5-fold com-

pared to the control (Figure 1). When the sandy soil was treated with FV + B10 and FV + B20 the CO_2 emissions by 4 and 8%, respectively, in comparison to soil that received F V ($P < 0.05$). The FV + B50 application decreased the CO_2 emissions by 11% compared to FV + B20 application ($P < 0.05$). In clayey soil, the FV treatment increased CO_2 emissions by 2.4-fold in comparison to the control (Figure 1). In addition, soils with FV + B10 increased other 6.4% the CO_2 emissions in comparison to FV. In contrast, soil that received FV + B20 and FV + B50 decreased the CO_2 emissions by 11% and 8%, respectively, compared to FV + B10 ($P < 0.05$).

The addition of filter cake to soil has been shown to increase CO_2 efflux. [19] found that filter cake amendment resulted in a 100-fold increased in CO_2 emissions compared to the unamended soil (control), likely due to the immediate utilization of labile sugars present in this residue. However, non-significant emissions of N applied as filter cake have been found in previous studies [5] [7].

Besides the high biochemical oxygen demand of vinasse, which causes a temporally reduced environment after its application to soil [35], the high amounts of bioavailable C in this residue can also fuel nitrification and denitrification processes. In plant cane, [5] observed that vinasse was associated with an increase in N_2O emissions of about 1060 kg CO_2-eq·ha^{-1}·yr^{-1}, and with an increase in CO_2 emissions of about 965 kg CO_2-eq·ha^{-1}·yr^{-1} compared to the mineral fertilizer plots.

The slight increase in cumulative CO_2 emissions with biochar amendment at 10 and 20 Mg·ha^{-1} may be attributed to the mineralization of easily available biochar-C at early stages of incubation. The labile C compounds of biochar combined with the high pH of this material (Table 1) may cause rapid changes in microbial activity [44] when applied to soil, and stimulate fast growing (r-strategists) microbes that are adapted to respond quickly to newly available C

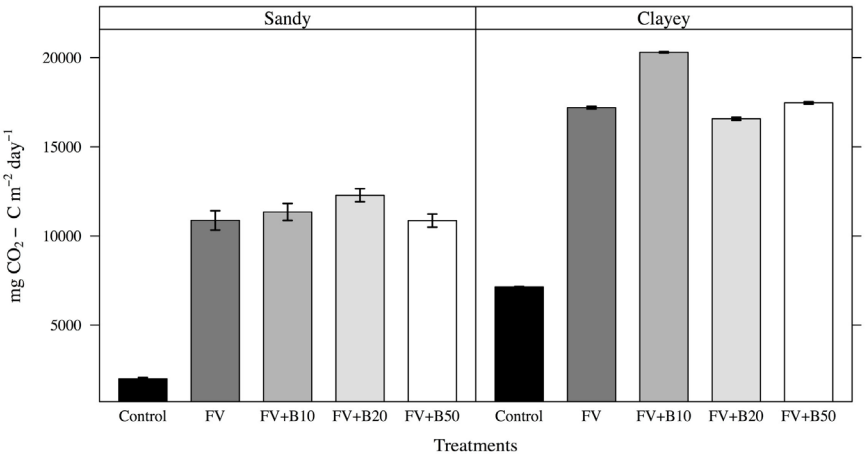

Figure 1. Mean cumulative fluxes of CO_2 measured over the incubation period (100 days) in sandy and clayey soil matrixes. Error bars are standard deviation (SD). The treatments are: soil with filter cake and vinasse (FV); plus biochar at 10 (FV + B10), 20 (FV + B20) and 50 (FV + B50) Mg·ha^{-1}.

sources, thereby increasing biochar-C mineralization [45] [46]. However, this phenomenon tends to decrease in the short-term due to the depletion of labile SOC [16].

An interesting outcome from both incubated sandy and clayey soils was that the highest biochar rate to soil dropped CO_2 emissions down to the levels comparable to vinasse and filter cake amendment. Although the understanding of the stability biochar C in soil has improved in recent years [16] [45] [46], there is a lack of knowledge about how both the soil- and biochar-C mineralization are affected as a function of biochar amount applied to soil.

According to [47], composting with biochar caused a positive priming (increased C mineralization) on non-biochar composting material at low (up to 1w%) biochar concentrations, while at high (up to 50w%) biochar concentrations negative priming (decreased non-biochar C mineralization) was observed. Moreover, [48] reported that the amount of biochar added to soil is inversely proportional to the impact of priming effect on C abatement potential.

The FV treatment also increased the N_2O emissions from the soils (by 5-fold in the case of the sandy soil and by 125% in the case of clayey soil) in comparison to the control (**Figure 2**). In contrast, increase in biochar applications decreased the N_2O emissions by 24% (FV + B10) and by 34% (FV + B50) in sandy soil, and by 14% (FV + B10) and 56% (FV + B50) in clayey soil in comparison to FV application only ($P < 0.05$).

[6] observed significantly higher N_2O emissions in the first days after vinasse application to sugarcane fields. 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.

The mechanisms by which some biochars could induce mitigation of soil N_2O

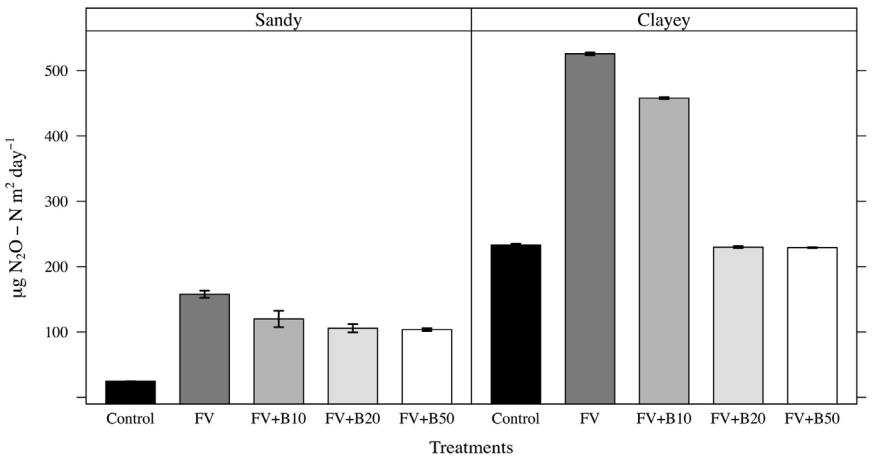

Figure 2. Mean cumulative fluxes of N_2O measured over the incubation period (100 days) in sandy and clayey soil matrixes. Error bars are standard deviation (SD). The treatments are: soil with filter cake and vinasse (FV); plus biochar at 10 (FV + B10), 20 (FV + B20) and 50 (FV + B50) $Mg{\cdot}ha^{-1}$.

emissions remain elusive [12] [13], and will most likely be a function of the biochar, soil properties and their interaction. Primarily, the feedstock from which biochar is produced, in particular its chemical (e.g. available N, 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.

Also, biochar application to 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 significant decrease in N_2O emissions as a function of biochar application rate, especially in clayey soil, could have been favored by increased soil aeration, which in turn reduced anaerobic microsites that favourdenitrification.

Finally, the CH_4 fluxes from the both soils (*i.e.*, sandy and clayey soils) were negligible (0.01 μg CH_4 - $C{\cdot}m^{-2}{\cdot}day^{-1}$), and did not showed significant effects of treatments and of soil matrixes (data not showed).

3.3. Bacterial Communities Shifts upon Biochar Addiction to Soil

The Permanova analysis revealed that the distribution of bacterial communities in both sandy and clayey soils was influenced by the experimental duration, application rates of biochar, and the interaction of these factors (**Table 4**). Moreover, the PCoA of DGGE band patterns showed higher temporal changes in bacterial community structure comparing 30 and 100 days of incubation in sandy soil (R = 0.70, $p = 0.0001$) (**Figure 3**), while clayey soil showed barely separable bacterial groups (R = 0.24, $p = 0.0001$) (**Figure 4**).

As abovementioned, biochar application to soil might be a pathway of microbial selection and activity [49]. The large surface area and amount of pores in biochar create new niches of microbe colonization, favouring the shifts in bac-

Table 4. PERMANOVA analyses testing for differences in the bacterial communities between incubation time (Days), application rates of biochar and their interaction (Days x biochar rate) in sandy and clayey soils.

Source	SS	df	MS	F
		Sandy soil		
Days	0.656	3	0.219	2.241*
Biochar rate	0.331	4	0.083	0.847*
Days x Biochar rate	0.106	12	0.009	0.091*
		Clayey soil		
Days	0.958	2	0.479	5.892*
Biochar rate	0.694	4	0.174	2.136*
Days x Biochar rate	1.488	8	0.186	2.288*

*p-value < 0.001.

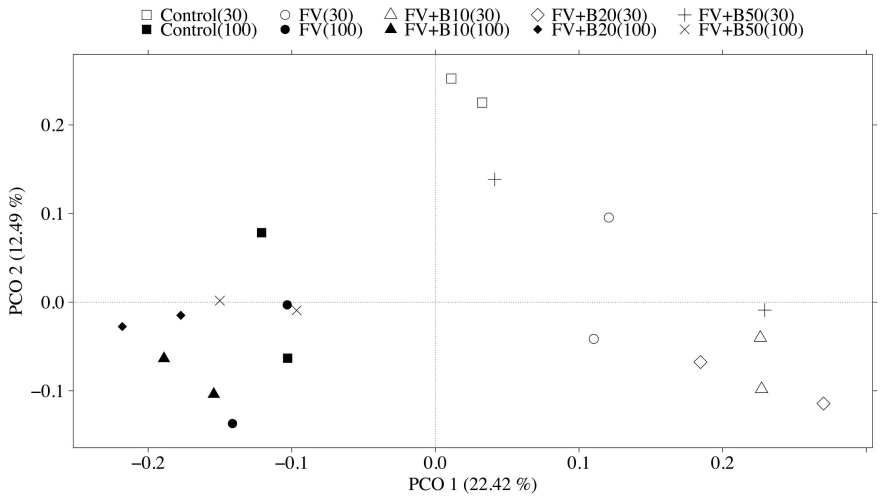

Figure 3. Principal coordinates analysis (PCoA) of the bacterial community distribution in sandy soil at 30 and 100 days of incubation. The treatments are: soil with filter cake and vinasse (FV); plus biochar at 10 (FV + B10), 20 (FV + B20) and 50 (FV + B50) $Mg{\cdot}ha^{-1}$.

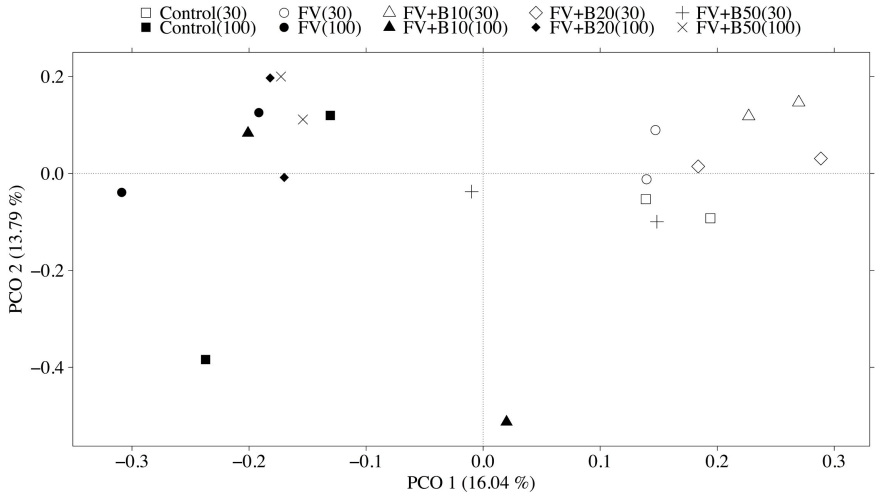

Figure 4. Principal coordinates analysis (PCoA) of the bacterial community distribution in clayey soil at 30 and 100 days of incubation. The treatments are: soil with filter cake and vinasse (FV); plus biochar at 10 (FV + B10), 20 (FV + B20) and 50 (FV + B50) $Mg{\cdot}ha^{-1}$.

terial community according to the amount [50], type [51] and persistence of the biochar applied to soil [40] [52].

According to [52], biochar have prompted the "charosphere", a region that surrounds its surface and it is permeated by many physical and chemical reactions, thus affecting soil pH, release of soluble C and nutrients availability, which may differentially influence the soil bacterial structure and composition. Clustering the results of bacterial communities, mineral N and N_2O emissions, it can be seen that the similar bacterial structure at 30 and 100 days of incubation in clayey soil (R = 0.24, $p = 0.0001$) was concomitant with decreasing NH_4^+-N and

NO_3^--N concentrations (Table 3) and N_2O emissions (Figure 2).

These results suggest that the interactions between biochar and the microbial community may drive the mitigation of GHG emissions, mainly N_2O. Most important, it seems that N_2O emission mitigation in clayey soil is more directly related to biotic mechanisms (*i.e.* direct changes in microbial community composition through biochar addition to soil); while in sandy soil the abiotic mechanisms caused by biochar (e.g. acid neutralizing capacity, cation exchange properties) play a more important role in reducing N_2O emissions, which in turn indirectly "activate" soil microbial communities to further reduce N_2O.

[53] showed the influence of biochar on temporal changes in bacterial community–either promoting an increase in abundance or reducing the magnitude of loss of species, a negative effect on bacterial abundance, and changes in the N cycling. The same authors showed that transcription factor peaks were closely related to bacterial groups such as Mycobacterium, which could play a crucial role in NO_3^- reduction, and Bradyrhizobium, reducing N_2O to N_2. Therefore, this could be a mechanism to explain the mitigation of N_2O emissions observed when biochar is applied to soil [54].

4. Conclusion

Biochar combined with filter cake and vinasse presented synergistic effects on soil pH, availability of P and exchangeable bases contents. However, the effects of this combination on soil amelioration were closely related to the soil buffering capacity, 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. Soil-biochar interactions caused temporal changes in bacterial communities both directly and indirectly, affecting niche-microbe interactions related to N_2O emission mitigation. Thus, there was a significant supression of N_2O emissions in contrasting soils treated with vinasse and filter cake as a function of biochar application rate.

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