The Open Agriculture Journal
DOI: 10.2174/0118743315362340241227050432, 2024, 18, e18743315362340
ISSN: 1874-3315

RESEARCH ARTICLE **OPEN ACCESS**

Greenhouse Gas Emissions and Ryegrass Yield after Application of Solid-Liquid Pig Slurry and Biochar to an Agricultural Soil

José L. S. Pereira[1,2,3,*], Adelaide Perdigão[1,2,3], Gabriel Bonifácio[1], Vitor Figueiredo[1], Francisco Marques[1,2], Henrique Trindade[3] and Dulcineia F. Wessel[1,2,4]

[1]*Agrarian Higher School of Viseu, Polytechnic Institute of Viseu, Quinta da Alagoa, 3500-606 Viseu, Portugal*
[2]*CERNAS-IPV Research Centre, Polytechnic Institute of Viseu, Campus Politécnico, Repeses, 3504-510 Viseu, Portugal*
[3]*Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes and Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal*
[4]*LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193Aveiro, Portugal*

Abstract:

Background: The application of animal slurry to the soil improves its quality, as manure contains many nutrients for plants. However, this could negatively impact the environment.

Objective: This field study investigated the effects of the addition of biochar after the mechanical separation of Whole pig Slurry (WS) into Solid (SF) and Liquid Fractions (LF) on Greenhouse Gases (GHG) emissions (N_2O, CO_2, and CH_4) and ryegrass (*Lolium multiflorum* Lam. cv magnum) yield.

Methods: Biochar (1.0 kg m^{-2}) was applied in plots alone or together with each of the three slurries (80 kg N ha^{-1}) in a total of eight treatments with three replications, including just soil with and without biochar as controls. Soil properties, Greenhouse Gas (GHG) fluxes, and yield were measured during theautumn/winter growing season.

Results: The results showed that the addition of biochar to these three slurries significantly increased the soil pH and showed no impact on the other physicochemical properties. The GHG emissions were not significantly different between treatments with and without biochar. The N use efficiency increased significantly in SF > WS > LF, whereas no differences were observed among these three slurries with and without biochar.

Conclusion: It can be concluded that the addition of biochar combined with WS or SF/LF to sandy-loam soil appears to have no impact on GHG emissions and ryegrass yield during the autumn/winter season. Overall, this finding suggests that amounts higher than 1.0 kg m^{-2} of biochar combined with SF may need to be applied to soil to reduce GHG emissions and nitrate leaching and increase N use efficiency and crop yield.

Keywords: Biochar, GHG emissions, Pig slurry, Solid-liquid separation, Ryegrass.

*Address correspondence to this author at the Agrarian Higher School of Viseu, Polytechnic Institute of Viseu, Quinta da Alagoa, 3500-606 Viseu, Portugal, CERNAS-IPV Research Centre, Polytechnic Institute of Viseu, Campus Politécnico, Repeses, 3504-510 Viseu, Portugal and Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes and Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal; Tel: +351232446600; Fax: +351232426536; E-mail: jlpereira@esav.ipv.pt

Cite as: Pereira J, Perdigão A, Bonifácio G, Figueiredo V, Marques F, Trindade H, Wessel D. Greenhouse Gas Emissions and Ryegrass Yield after Application of Solid-Liquid Pig Slurry and Biochar to an Agricultural Soil. Open Agric J, 2024; 18: e18743315362340. http://dx.doi.org/10.2174/0118743315362340241227050432

Received: October 05, 2024
Revised: November 20, 2024
Accepted: December 02, 2024
Published: December 31, 2024

1. INTRODUCTION

Globally, animal production has tended to rely on more intensive practices, resulting in increasing volumes of animal slurry (liquid manure). Scarlat *et al*. reported that, in the EU-28, around 1.3 billion tons of manure are produced annually from 89.5 million bovines, 147.8 million pigs, and 1.7 billion poultry [1]. Crop fertilization with animal slurries has a long tradition as a way of closing nutrient cycles on farms, emphasizing the concept of circular economy.

With the increase in the amount of animal slurry produced, environmental concerns have risen in recent years [2, 3]. Animal manure needs to be used efficiently, promoting agricultural soil fertility, protecting the environment (emissions into the atmosphere and leaching into the water system), and, finally, contributing to global health at the human-animal-ecosystem interface [4]. With the increase of slurry produced from animal farming, the monitoring and mitigation of Greenhouse Gases (GHG) and ammonia (NH_3) emissions represent a major issue [5]. The two major GHGs emitted by the agriculture/livestock sector are methane (CH_4) and nitrous oxide (N_2O). The European Union (EU) climate and energy framework has committed to reducing GHG emissions from animal waste and agriculture by 30% below 2005 levels in 2030, as stated in Regulation EU 2018/842.

The application of animal slurry to the soil improves its quality and reduces the use of mineral fertilizers and production costs, as it contains essential nutrients for crop growth [6]. Consequently, the use of slurries as a fertilizer is a sustainable agricultural practice that allows one to recycle nutrients that would otherwise be lost to the atmosphere and water. The mechanical separation of slurry is an adequate management technique of the manure that allows the separation of the Liquid Fraction (LF), rich in ammoniacal nitrogen (NH_4^+) and potassium (K), from the Solid Fraction (SF), rich in organic matter, P and relatively rich in nitrogen (N) [7].

Biochar, as a soil amendment, has shown potential for mitigating gaseous emissions, and its beneficial role in the improvement of soil quality is widely reported, enhancing crop yield and carbon (C) sequestration, particularly under adverse climatic conditions [8, 9, 10]. It is considered the easiest and most widely usable tool to increase soil C stocks [11-14]. The mechanisms through which biochar influences GHG emission are modification of soil aeration, water holding capacity, adsorption, pH, available nutrients, and activity of soil microbes and enzymes [15]. Despite the positive effects of biochar addition to soil, there is a gap in knowledge since previous studies are not conclusive about the effects of biochar in combination with both inorganic and organic fertilizers on climate conditions, soil type, nutrient availability, and use efficiency, crop productivity, mitigation of GHG emissions, and nutrient leaching [16-21].

The aim of this field study was to assess the effect of the addition of biochar after the mechanical separation of whole pig slurry on N_2O, CO_2, and CH_4 emissions and ryegrass (*Lolium multiflorum* Lam. cv magnum) yield from solid and liquid fractions.

2. MATERIALS AND METHODS

2.1. Location and Slurry Management

An experimental field was established from October 2019 to June 2020 at the Agrarian Higher School of Viseu (Viseu, Portugal; latitude: 40.641789º, longitude: -8.655840º). The long-term yearly mean air temperature in the region was 14.2 ºC, the monthly mean air minimum was 6.9 ºC in January, and the maximum was 21.4 ºC in July. The long-term average annual rainfall in the region was 1200 mm. The highest average monthly precipitation was recorded in December, with 204 mm. The average monthly temperatures and amounts of precipitation during this experiment were recorded by an automatic compact weather station (WS-GP1, Delta-T Devices Ltd, UK) and are presented in Table **1**.

The soil used in this study was classified as Dystric Fluvisol [22], with a sandy-loam texture (44.2% coarse sand, 24.1% fine sand, 16.3% silt, and 15.4% clay). The physicochemical properties of the soil were determined by standard laboratory methods [19], with the following values: bulk density, 0.9 g cm^{-3}, pH (H_2O), 6.0, electrical conductivity, 0.02 mS cm^{-1}, water holding capacity (WHC) at pF 2.0, 38.4% (w/w), total organic C, 15.60 g kg dry $soil^{-1}$, and total N, 1.84 g kg dry $soil^{-1}$.

Table 1. Climate data recorded from the weather station during the experiment (mean ± standard deviation).

Month	Soil Temperature (ºC)	Air Temperature (ºC)	Relative Humidity (%)	Cumulative Rainfall (mm)
October 2019	18.7 ± 1.1	13.8 ± 1.3	83.2 ± 4.2	136.2
November 2019	18.9 ± 1.4	10.1 ± 1.3	90.8 ± 2.1	260.6
December 2019	17.3 ± 1.0	9.8 ± 0.9	83.0 ± 6.2	336.1
January 2020	14.8 ± 0.8	8.6 ± 1.3	83.2 ± 5.6	121.7
February 2020	17.2 ± 0.8	11.3 ± 0.9	78.4 ± 6.9	35.5
March 2020	17.7 ± 0.8	11.4 ± 1.4	74.5 ± 5.8	124.2
April 2020	17.7 ± 0.4	13.2 ± 0.9	85.0 ± 4.0	154.1
May 2020	23.5 ± 1.2	18.8 ± 1.8	72.7 ± 5.0	49.7
June 2020	23.6 ± 1.2	18.4 ± 1.6	73.1 ± 4.1	6.2

Table 2. Physicochemical properties of the slurries used and amounts applied to the experiment (mean ± standard deviation).

Parameters	Whole Slurry (WS)	Solid Fraction (SF)	Liquid Fraction (LF)
Proportion (% of raw slurry)	100 ± 1 a	20 ± 1 c	80 ± 1 b
pH (H_2O)	7.8 ± 0.1 b	7.9 ± 0.1 b	8.6 ± 0.1 a
Dry matter (g kg^{-1})	7.2 ± 2.7 b	383.3 ± 5.3 a	6.4 ± 0.9 b
Total C (g kg^{-1})	33.7 ± 3.8 b	53.5 ± 4.5 a	17.4 ± 0.1 c
Total N (g kg^{-1})	2.8 ± 0.1 b	3.1±0.1 a	2.6 ± 0.1 b
NH_4^+-N (g N kg^{-1})	2.5 ± 0.1 a	2.3 ± 0.1 a	2.4 ± 0.1 a
NO_3^--N (mg N kg^{-1})	7 ± 1 b	26 ± 4 a	8 ± 1 b
NH_4^+: total N ratio	0.89 ± 0.01 a	0.74 ± 0.01 b	0.92 ± 0.01 a
C/N ratio	12 ± 1 b	18 ± 2 a	7 ± 1 c
Application rate	-	-	-
kg C ha^{-1}	969 ± 220 b	1406 ± 298 a	543 ± 6 c
kg N ha^{-1}	80 ± 1 a	80 ± 1 a	80 ± 1 a
kg NH_4^+-N ha^{-1}	71 ± 1 a	60 ± 1 b	74 ± 1 a

Note: Data expressed on a fresh-weight basis. Values presented with different lowercase letters within rows are significantly different ($p < 0.05$) by Tukey test. $n = 3$: three replications per parameter.

The pig slurry used in this study came from a local farm. The whole slurry was subjected to mechanical separation by sieving through a 1.0 mm screen, generating a Solid Fraction (SF) and a Liquid Fraction (LF), with the following separation yields (w/w): 20% for SF and 80% for LF. The three slurries were subsampled in triplicate and analyzed by standard laboratory methods for the physicochemical properties detailed in Table **2** [6]. The soil texture was determined with the international pipette, soil bulk density by the Keen & Raczkowski method, pH (H_2O) by potentiometry in a 1:2.5 soil: water ratio for soil and directly for slurry, water holding capacity by the gravimetric method, total C by the Dumas method, total N by the Kjeldahl method, and NH_4^+ and NO_3^- by spectrophotometry.

2.2. Experimental Details

The experiment was a randomized complete block design with three replicates and eight treatments. Field plots measuring 3.0 m x 2.0 m each were established and assigned treatments, totaling twenty-four plots. Three slurries (WS, SF, and LF) and control were considered in combination with and without biochar addition. Thus, the eight treatments considered were the following:

[1] Non-amended soil without and with biochar (Control and Biochar treatments),
[2] Application of whole slurry without and with biochar (WS and WS+Biochar treatments),
[3] Application of the solid fraction without and with biochar (SF and SF+Biochar treatments),
[4] Application of the liquid fraction without and with biochar (LF and LF+Biochar treatments).

After preparing the field soil by ploughing and discing, on the 20th of October 2019, WS, SF, and LF were manually applied to the soil of each designated plot at a rate of 80 kg N ha^{-1}. Then, in each designated plot, biochar was applied manually at a rate of 1.0 kg m^{-2} [16, 19]. All soil plots were immediately scraped manually (20 mm depth) to incorporate the treatments and prevent NH_3 volatilization from the slurries. Ryegrass (*Lolium multiflorum Lam. cv magnum*) was sown by hand the following day (21st October 2019) at a density of 35 kg ha^{-1} as used by local farmers. Ryegrass was rainfed, and no weed control was performed.

The commercial biochar (Ibero Massa Florestal, S.A., Portugal) was obtained from wood (agroforestry tree species) shavings (Ø = 2 mm) pyrolyzed in a muffle furnace at 900 °C. The main physicochemical properties of the biochar were determined by standard laboratory methods [19], with the following values: pH (H_2O), 9.9; dry matter, 897.6 g kg^{-1}; total C, 782.5 g kg^{-1}; total N, 2.0 g kg^{-1}; average particle size, 21 µm; 90% size of particles, > 37 µm; specific surface area, 22 m^2 g^{-1}; and pore volume, 1.1 mm^3 g^{-1}. Briefly, the biochar pH (H_2O) was determined by potentiometry, dry matter by the gravimetric method, total C by the Dumas method, total N by the Kjeldahl method, particle size by the sieving method, specific surface area by the Brunauer, Emmett, and Teller method, and pore volume by mercury porosimetry.

2.3. Soil Mineral N and Crop Yield

Soil mineral N was determined in the 0-200 mm layer, 1, 3, 5, 7, 14, 21, 30, 60, 90, 120, and 150 days after the beginning of the experiment. A composite sample per plot was taken (six replicates), mixed, sieved (2 mm), and frozen (-18 ºC). A soil subsample was dried at 105 ºC to constant weight for gravimetric water content determination. Another subsample was used for pH determination. Then, the soil samples frozen were analyzed for NH_4^+ and NO_3^- concentrations by automated segmented flow spectrophotometry (San Plus, Skalar,

Breda, The Netherlands) after extraction with 2 M KCl (1:5 w/v) and filtration (Whatman 42).

On the 7th of May 2020, the yield of the aboveground biomasses of ryegrass was obtained by cutting the crop to a height of 50 mm from 0.25 m^2 in each plot and weighing it. The aliquot subsamples of the ryegrass were used to determine Dry Matter (DM) yields by drying to a constant mass at 65 ºC in a forced-draught oven. The N content in the samples was determined using the Kjeldahl method. Nitrogen uptake was determined by multiplying dry matter weight (aboveground biomass) by N content. The Apparent N Recovery (ANR) and N Use Efficiency (NUE) were calculated using the Eqs. (**1** and **2**), respectively, as mentioned below [7, 23]:

$$ANR = \frac{(NU_T - NU_C)}{N_A} \quad (1)$$

where, *ANR* isthe apparent N recovery in each amended treatment (g g^{-1}), NU_T is the N in the DM yield obtained with the amendment treatment, NU_C isthe N in the DM yield obtained with the Control treatment, and N_A is referred to the N provided by the slurries.

$$NUE = \frac{(DM_T - DM_C)}{N_A} \quad (2)$$

where, *NUE* isthe N use efficiency in each amended treatment (g DM g^{-1} N), DM_T is the DM yield obtained with the amendment treatment, DM_C is the DM yield obtained with the Control treatment, and N_A is referred to the N provided by the slurries.

2.4. Gas Flux Measurements

Fluxes of N_2O, CO_2, and CH_4 were measured using the closed chamber technique and following the procedure described in Fangueiro *et al*. [6]. Gas measurements were carried out 1, 2, 3, 6, 7, 8, and 9 days after the experiment's beginning, twice a week until day 30, once a week until day 60, and twice a month at the end of the experiment. To evaluate the GHG gas fluxes from each treatment, a circular chamber of polyvinyl chloride (Ø = 200 mm, h = 110 mm), equipped with a septum to sample the interior atmosphere, was inserted into the soil (depth = 30 mm). The chambers were kept at fixed locations throughout the sampling dates. After the chamber was closed, a first gas sample (25 mL) was taken (t = 0.0 h) using a plastic syringe and flushed through gas vials (20 mL), then a second (t = 0.5 h) and a third (t = 1.0 h) gas sample was taken from the headspace of the chamber and stored in vials [6]. The concentrations of the gas samples stored in vials were measured by gas chromatography using a GC-2014 (Shimadzu, Japan) equipped with a Thermal Conductivity Detector (TCD) for CO_2 and an electron capture ^{63}Ni detector (ECD) for N_2O. The GC-2014 accuracy was 1 ppm to 1% for CO_2 and 50 ppb to 100 ppm for N_2O. The N_2O, CO_2, and CH_4 fluxes were determined using Eq. (**3**), given as follows [6]:

$$\text{FLUX} = \frac{CONC \times MOLE}{IDEA \times \left(\frac{273+TEMP}{273}\right)} \times HEIG \times TIME \quad (3)$$

where, *FLUX* is the N_2O, CH_4, or CO_2 flux on each sampling date (g N or C m^{-2} day^{-1}), *CONC* is the gas concentration (m^3 m^{-3}), *MOLE* is the gas molecular weight (44 g mol^{-1} for N_2O or CO_2 and 16 g mol^{-1} for CH_4), *IDEA is* the volume of an ideal gas (0.0224 m^3 mol^{-1}), *TEMP* is the temperature during the sampling period (ºC), *HEIG* is the height of the chamber (0.080 m), and *TIME* isthe time corrected per day.

To calculate cumulative gas emissions, the flux between two sampling occasions was averaged and then multiplied by the time interval between the measurements. The conversion factors of 265 for N_2O and 28 for CH_4 were used to express the Global Warming Potential (GWP) [24] as CO_2 -equivalents, using Eq. (**4**), given as follow:

$$YSG = \frac{(265 \times \sum N2O) + (28 \times \sum CH4)}{YIE} \quad (4)$$

where, *YSG* is the net GWP per unit of ryegrass yield (g CO_2-eq g^{-1}), ΣN_2O and ΣCH_4 are the accumulated amounts of N_2O and CH_4 released during ryegrass cropping (g CO_2-eq m^{-2}), and *YIE* is the ryegrass yield (g m^{-2}).

The N_2O, CO_2, CH_4, and GWP losses from amended treatments areexpressed as reduction efficiencies [24] using Eq. (**5**), as follows:

$$REDUC = 100 - \left(\left(\frac{TREAT}{CONTR}\right) \times 100\right), \quad (5)$$

where, *REDUC* isthe reduction efficiency from each amended treatment relative to the Control treatment (%), *TREAT* isthe mean value of the individual/cumulative gas loss from each amended treatment, and *CONTR* isthe mean value of the individual/cumulative gas loss from the Control treatment.

2.5. Statistical Analysis

Analysis of variance was conductedusing the statistical software package STATISTIX 10 (Analytical Software, Tallahassee, FL, USA) to assess the effect of slurries, biochar, and slurries × biochar interaction. The Shapiro-Wilk normality test was used to determine the normality of the analyzed traits' distribution [25, 26]. The collected data was analyxed per day, and for the whole experiment, a randomized complete block design was considered using two factors: slurries and biochar. Tukey comparisons of means ($p < 0.05$) were carried out for the factors and their interactions [27].

3. RESULTS

3.1. Soil Properties

The concentrations of NH_4^+ in the soil of each treatment are presented in Table **3** and were lower than 11 mg NH_4^+-N kg^{-1} of dry soil in the control and biochar treatments during the 195 days of the experiment. In the first 6 days of the study, NH_4^+ concentrations increased

significantly ($p < 0.05$) in treatments that received slurries (WS, SF and LF), without and with biochar (WS+Biochar, SF+Biochar and LF+Biochar), when compared to treatments without slurries (control and biochar), with concentrations that ranged from 27 to 75 mg NH_4^+-N kg^{-1} of dry soil being observed (Table **3**). From day 14 until the end of the experiment, the NH_4^+ concentrations did not differ significantly ($p > 0.05$) between treatments without and with slurries, and they declined to background levels (9 to 3 mg NH_4^+-N kg^{-1} of dry soil) by the nitrification process (Table **3**). The NH_4^+ concentrations in treatments with and without biochar did not differ significantly ($p > 0.05$) during the experiment, although numerically higher values were observed in some measurements of treatments with biochar (Table **3**).

The initial concentrations of NO_3^- in the control and biochar treatments were low and remained constant until the end of the experiment (Table **4**). Compared to treatments without slurries, an increase in NO_3^- concentrations was observed in treatments that received slurries with and without biochar, with a peak observed on day 6 (15 to 22 mg NO_3^--N kg^{-1} of dry soil) followed by a decrease to background levels by the end of the experiment (Table **4**). In most measurement days, no significant differences ($p > 0.05$) of NO_3^- concentrations between all treatments with and without biochar were observed (Table **4**).

Table 3. Soil concentrations of NH_4^+ observed in treatments of the experiment (mean ± standard deviation).

-	Days of Experiment								
Treatments	Day 1	Day 3	Day 6	Day 14	Day 22	Day 37	Day 76	Day 120	Day 195
-	(mg NH_4^+-N kg^{-1} dry soil)								
Control	4 ± 2 e	9 ± 1 b	4 ± 1 c	6 ± 3 a	7 ± 1 a	3 ± 1 a	5 ± 1 c	9 ± 2 a	3 ± 1 a
Biochar	11 ± 3 de	10 ± 1 b	4 ± 1 c	6 ± 2 a	8 ± 1 a	4 ± 1 a	9 ± 1 abc	8 ± 2 a	2 ± 1 a
WS	66 ± 20 ab	49 ± 14 a	33 ± 1 b	9 ± 6 a	16 ± 3 a	6 ± 1 a	9 ± 2 abc	6 ± 1 a	2 ± 1 a
WS+Biochar	69 ± 26 a	58 ± 2 a	75 ± 18 a	3 ± 2 a	20 ± 9 a	6 ± 1 a	9 ± 1 abc	7 ± 1 a	3 ± 1 a
SF	27 ± 9 cd	43 ± 12 a	35 ± 6 b	7 ± 5 a	12 ± 4 a	5 ± 1a	11 ± 1 a	6 ± 1 a	3 ± 1 a
SF+Biochar	18 ± 4 cd	46 ± 3 a	35 ± 5 b	2 ± 1 a	10 ± 1 a	6 ± 1 a	9 ± 1 abc	7 ± 1 a	3 ± 1 a
LF	56 ± 17 bc	52 ± 11 a	31 ± 13 b	3 ± 2 a	17 ± 4 a	5 ± 1 a	5 ± 1 bc	7 ± 1 a	3 ± 1 a
LF+Biochar	48 ± 7 bcd	50 ± 5 a	38 ± 11 b	7 ± 6 a	12 ± 1 a	4 ± 1 a	10 ± 3 ab	7 ± 1 a	3 ± 1 a
p slurries (A)	***	***	***	ns	ns	ns	ns	ns	ns
p biochar (B)	ns	ns	*	ns	ns	ns	ns	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values from the interaction of slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01 and 0.001 probability level, respectively. $n = 3$: three replications per treatment.

Table 4. Soil concentrations of NO_3^- observed in treatments of the experiment (mean ± standard deviation).

-	Days of Experiment								
Treatments	Day 1	Day 3	Day 6	Day 14	Day 22	Day 37	Day 76	Day 120	Day 195
-	(mg NO_3^--N kg^{-1} dry soil)								
Control	2 ± 1 b	9 ± 4 ab	6 ± 3 b	20 ± 6 a	4 ± 1 b	1 ± 1 b	2 ± 1 ab	1 ± 1 ab	1 ± 1 ab
Biochar	5 ± 2 ab	6 ± 1 b	9 ± 5 ab	7 ± 1 ab	2 ± 1 b	3 ± 1 ab	1 ± 1 ab	1 ± 1 ab	1 ± 1 b
WS	7 ± 2 a	9 ± 1 ab	17 ± 2 ab	7 ± 5 ab	27 ± 8 a	2 ± 1 ab	1 ± 1 b	3 ± 2 a	1 ± 1 ab
WS+Biochar	6 ± 2 ab	8 ± 1 ab	15 ± 2 ab	4 ± 2 b	12 ± 9 ab	1 ± 1 b	1 ± 1 b	1 ± 1 ab	1 ± 1 ab
SF	5 ± 2 ab	15 ± 3 a	19 ± 3 ab	10 ± 2 ab	3 ± 1 b	3 ± 1 ab	3 ± 1 a	1 ± 1 ab	1 ± 1 a
SF+Biochar	4 ± 1 ab	14 ± 2 a	21 ± 4 a	8 ± 4 ab	5 ± 2 b	3 ± 1 ab	1 ± 1 ab	1 ± 1 ab	1 ± 1 ab
LF	4 ± 1 ab	13 ± 1 a	18 ± 6 ab	9 ± 3 ab	11 ± 5 ab	1 ± 1 b	2 ± 1 ab	1 ± 1 b	1 ± 1 b
LF+Biochar	4 ± 1 ab	14 ± 2 a	22 ± 4 a	10 ± 6 ab	20 ± 10 ab	4 ± 1 a	1 ± 1 b	1 ± 1 b	1 ± 1 a
p slurries (A)	ns	*	*	ns	*	ns	ns	ns	ns
p biochar (B)	ns	ns	ns	ns	ns	ns	ns	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values from the interaction slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01 and 0.001 probability level, respectively. $n = 3$: three replications per treatment.

Table 5. Soil pH (H_2O) observed in treatments of the experiment (mean ± standard deviation).

-	Days of Experiment								
Treatments	Day 1	Day 3	Day 6	Day 14	Day 22	Day 37	Day 76	Day 120	Day 195
Control	6.6 ± 0.1 b	6.1 ± 0.1 d	6.4 ± 0.1 b	6.4 ± 0.1 a	6.5 ± 0.1 a	6.9 ± 0.1 a	6.6 ± 0.1 b	6.5 ± 0.1 ab	5.9 ± 0.2 b
Biochar	6.8 ± 0.1 b	6.6 ± 0.1 abc	6.7 ± 0.1 ab	6.5 ± 0.1 a	6.6 ± 0.1 a	6.9 ± 0.1 a	6.7 ± 0.1 ab	6.4 ± 0.1 ab	6.4 ± 0.1 a
WS	6.5 ± 0.1 ab	6.4 ± 0.1 cd	6.6 ± 0.1 ab	6.5 ± 0.1 a	6.6 ± 0.1 a	6.9 ± 0.1 a	6.5 ± 0.1 b	6.5 ± 0.1 a	5.7 ± 0.2 b
WS+Biochar	7.2 ± 0.3 a	6.8 ± 0.1 ab	6.9 ± 0.2 a	6.5 ± 0.1 a	6.7 ± 0.1 a	6.8 ± 0.1 a	6.6 ± 0.1 ab	6.4 ± 0.1 b	5.7 ± 0.1 b
SF	6.5 ± 0.1 b	6.4 ± 0.2 bc	6.4 ± 0.1 b	6.7 ± 0.2 a	6.7 ± 0.1 a	6.8 ± 0.1 a	6.7 ± 0.1 ab	6.4 ± 0.1 b	5.9 ± 0.1 ab
SF+Biochar	6.8 ± 0.1 ab	6.9 ± 0.1 a	6.7 ± 0.1 ab	6.5 ± 0.1 a	6.7 ± 0.1 a	7.0 ± 0.2 a	6.7 ± 0.1 ab	6.4 ± 0.1 b	5.8 ± 0.3 b
LF	6.7 ± 0.1 ab	6.6 ± 0.1 abc	6.5 ± 0.1 b	6.6 ± 0.2 a	6.6 ± 0.1 a	7.0 ± 0.2 a	6.6 ± 0.1 b	6.3 ± 0.1 b	6.1 ± 0.1 ab
LF+Biochar	7.2 ± 0.3 a	6.5 ± 0.1 bc	6.8 ± 0.2 a	6.7 ± 0.1 a	6.7 ± 0.1 a	6.9 ± 0.1 a	6.8 ± 0.1 a	6.4 ± 0.1 b	6.0 ± 0.2 ab
p slurries (A)	ns	ns	ns	ns	ns	ns	ns	ns	ns
p biochar (B)	**	**	**	ns	ns	ns	*	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values from the interaction slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01, and 0.001 probability level, respectively. $n = 3$: three replications per treatment.

Table 6. Average N_2O emissions observed in treatments of the experiment (mean ± standard deviation).

-	Days of Experiment											
Treatments	Day 1	Day 2-3	Day 4-7	Day 8-18	Day 19-42	Day 43-74	Day 44-75	Day 76-121	Day 122-138	Day 139-195	∑0-195	∑0-195
-	(μg N_2O-N m^{-2} day^{-1})										(kg N_2O-N ha^{-1})	(% N applied)
Control	515 ± 172 a	97 ± 31 b	194 ± 34 a	319 ± 146 d	59 ± 17 b	108 ± 93 a	38 ± 33 a	6 ± 5 b	97 ± 43 a	46 ± 21 b	0.6 ± 0.1 c	-
Biochar	630 ± 51 a	86 ± 29 b	123 ± 73 a	427 ± 21 d	57 ± 17 b	51 ± 33 a	1 ± 1 a	51 ± 10 ab	1 ± 1 a	179 ± 9 a	0.5 ± 0.1 c	-
WS	1285 ± 667 a	1468 ± 676 a	489 ± 32 a	2867 ± 672 bcd	807 ± 268 a	1 ± 1 a	19 ± 10 a	60 ± 23 ab	53 ± 46 a	84 ± 39 b	2.5 ± 0.5 ab	3.1 ± 0.6 ab
WS+Biochar	1269 ± 228 a	866 ± 359 ab	739 ± 199 a	5436 ± 2458 ab	285 ± 153 b	32 ± 14 a	22 ± 14 a	101 ± 31 a	34 ± 30 a	63 ± 19 b	2.7 ± 0.9 ab	3.4 ± 1.2 ab
SF	985 ± 231 a	555 ± 241 ab	578 ± 299 a	2015 ± 713 cd	178 ± 93 b	12 ± 9 a	25 ± 21 a	36 ± 23 b	69 ± 42 a	32 ± 23 b	1.3 ± 0.4 bc	1.6 ± 0.5 b
SF+Biochar	752 ± 70 a	258 ± 125 ab	189 ± 10 a	2614 ± 350 bcd	56 ± 11 b	16 ± 14 a	13 ± 11 a	4 ± 4 b	17 ± 4 a	1 ± 1 b	1.1 ± 0.1 bc	1.4 ± 0.2 b
LF	1319 ± 282 a	878 ± 321 ab	680 ± 255 a	3798 ± 593 bc	130 ± 32 b	293 ± 253 a	9 ± 8 a	10 ± 9 b	27 ± 24 a	56 ± 37 b	1.9 ± 0.2 abc	2.3 ± 0.2 ab
LF+Biochar	1150 ± 247 a	1213 ± 420 ab	406 ± 206 a	7911 ± 963 a	419 ± 189 ab	4 ± 3 a	1 ± 1 a	4 ± 4 b	87 ± 41 a	21 ± 9 b	3.5 ± 0.5 a	4.3 ± 0.6 a
p slurries (A)	ns	*	ns	***	*	ns	ns	**	ns	*	***	ns
p biochar (B)	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns

Note: Values from the interaction slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01 and 0.001 probability level, respectively. n = 3: three replications per treatment.

The pH of the soil in the control treatment varied slowly (6.6 to 5.9) from the beginning to the end of the experiment (Table **5**). Compared to the control treatment, soil pH increased numerically ($p > 0.05$) in all other treatments during the first 6 days, followed by a decrease in control levels at the end of the experiment (Table **5**). Furthermore, soil pH increased in all treatments that received biochar, compared to the same treatments without biochar, but no significant variation ($p > 0.05$) was observed (Table **5**).

3.2. Greenhouse Gas Emissions

In the first 42 days of the experiment, the daily N_2O fluxes increased in all treatments relative to control and biochar treatments, followed by a similar pattern to these treatments until the end of the experiment (Table **6**). The first peak was observed in the first 3 days of the experiment (260-1470 μg N_2O-N m^{-2} day^{-1}), and the second peak reached in days 8-18 (2015-7911 μg N_2O-N m^{-2} day^{-1}) (Table **6**). Then, the N_2O fluxes decreased in all treatments

until the end of the experiment (Table **6**). In comparison to the WS and LF treatments, the N_2O fluxes from the SF treatments were reduced by ca. 26% during the first 42 days of the experiment (Table **6**). In most measurement days, no significant differences ($p > 0.05$) in N_2O fluxes between all treatments without and with biochar were observed, although numerically lower fluxes in SF treatment without biochar were observed (Table **6**). Compared to the control treatment, the cumulative N_2O emissions increased, but not significantly ($p > 0.05$), in treatments that received slurries by 393% for WS and 187% for SF/LF (Table **6**). Also, there were no significant differences ($p > 0.05$) in cumulative N_2O emissions, expressed as a percentage of N applied, from treatments that received slurries, although higher losses in WS treatment (3.1% for WS against 1.6% for SF) were observed (Table **6**). However, no significant differences ($p > 0.05$) in cumulative N_2O emissions between all treatments, expressed as absolute values or as a percentage of N applied, were observed (Table **6**).

The CO_2 daily fluxes increased in all treatments relative to control and biochar treatments, followed by a reduction until the end of the measurements (Table **7**). The first peak was observed on days 8-18 (5-11 g CO_2 m^{-2} day^{-1}) of the experiment, and the second peak was detected on days 122-138 (4-11 g CO_2 m^{-2} day^{-1}) (Table **7**). Compared to the WS and LF treatments, the CO_2 fluxes from the SF treatments reached an increase of ca. 50% in most measurements (Table **7**). No significant differences ($p > 0.05$) in CO_2 fluxes among all treatments without and with biochar were observed (Table **7**). The cumulative CO_2 emission of SF treatment was significantly higher ($p < 0.05$) by 100% than all treatments without biochar (Table **7**). The cumulative CO_2 emissions did not differ significantly ($p > 0.05$) among all treatments with and without biochar, although numerically higher values, around 60%, were observed for WS+Biochar and LF+Biochar treatments (Table **7**).

Measurable CH_4 fluxes were observed from the beginning until the end of the experiment, with values that varied from -1 to 11 mg CH_4 m^{-2} day^{-1} (Table **8**). Compared to control and biochar treatments, the peak was observed on days 8-18 (9–11 g CH_4 m^{-2} day^{-1}) in WS and WS+Biochar treatments, while all other treatments peaked (7-10-g CH_4 m^{-2} day^{-1}) on days 43-74 (Table **8**). In other measured dates, the daily fluxes of CH_4 did not differ significantly ($p > 0.05$) among treatments with and without biochar and followed a similar trend in the remaining measurements (Table **8**). The cumulative CH_4 emissions in treatments that received slurries were significantly higher ($p < 0.05$) than in control, with increases between 135 and 160% (Table **8**). No significant difference ($p > 0.05$) in cumulative CH_4 emissions between treatments with and without biochar was observed, although a numerical reduction of 37% was observed in the SF treatment compared to SF+Biochar (Table **8**).

The GWP, expressed as CO_2-equivalents, in the SF treatment was significantly higher ($p < 0.05$) than in control and WS/LF treatments, with increases between 135 and 160% (Table **9**). The cumulative GWP emissions were not significantly different ($p > 0.05$) between treatments with and without biochar, although a numerical reduction of 25% was observed in the SF treatment compared to SF+Biochar (Table **9**). The yield-scaled GWP was not significantly different ($p > 0.05$) between the control and treatments that received slurries, although it was numerically lower (-34 to -51%) in the WS/LF treatments (Table **9**). The yield-scaled GWP emissions were not significantly different ($p > 0.05$) between treatments with and without biochar, although a numerical reduction of 21% was observed in the SF+Biochar treatment compared to SF (Table **9**).

Table 7. Average CO_2 emissions observed in treatments of the experiment (mean ± standard deviation).

-	Days of Experiment										
Treatments	Day 1	Day 2-3	Day 4-7	Day 8-18	Day 19-42	Day 43-74	Day 44-75	Day 76-121	Day 122-138	Day 139-195	∑0-195
-	(g CO_2 m^{-2} day^{-1})										(ton CO_2 ha^{-1})
Control	2 ± 1 bc	2 ± 1 ab	3 ± 1 abc	5 ± 1 c	1 ± 1 b	1 ± 1 a	1 ± 1 ab	1 ± 1 b	6 ± 1 a	3 ± 1 a	18.9 ± 0.7 b
Biochar	1 ± 1 c	2 ± 1 b	2 ± 1 c	5 ± 1 c	1 ± 1 b	1 ± 1 a	1 ± 1 ab	1 ± 1 b	5 ± 1 a	3 ± 1 a	18.7 ± 4.1 b
WS	3 ± 1 ab	5 ± 2 a	3 ± 1 ab	5 ± 1 c	2 ± 1 ab	1 ± 1 a	1 ± 1 b	1 ± 1 b	4 ± 2 a	1 ± 1 a	15.8 ± 4.5 b
WS+Biochar	4 ± 1 a	3 ± 1 ab	3 ± 1 abc	6 ± 1 c	2 ± 1 ab	1 ± 1 a	2 ± 1 ab	3 ± 1 ab	8 ± 3 a	1 ± 1 a	25.4 ± 3.3 ab
SF	2 ± 1 abc	3 ± 2 ab	4 ± 1 a	10 ± 2 ab	4 ± 1 a	1 ± 1 a	1 ± 1 ab	5 ± 2 a	11 ± 3 a	3 ± 1 a	38.8 ± 3.5 a
SF+Biochar	2 ± 1 bc	3 ± 1 ab	2 ± 1 bc	11 ± 1 a	3 ± 1 ab	1 ± 1 a	1 ± 1 b	1 ± 1 b	10 ± 1 a	3 ± 2 a	28.9 ± 2.6 ab
LF	2 ± 1 abc	3 ± 1 ab	3 ± 1 abc	6 ± 1 c	2 ± 1 ab	1 ± 1 a	1 ± 1 b	2 ± 1 ab	5 ± 2 a	2 ± 1 a	19.5 ± 5.9 b
LF+Biochar	2 ± 1 bc	3 ± 1 ab	2 ± 1 c	7 ± 1 bc	3 ± 1 ab	1 ± 1 a	3 ± 2 a	1 ± 1 b	10 ± 3 a	4 ± 2 a	31.5 ± 9.6 ab
p slurries (A)	*	ns	ns	**	*	ns	ns	ns	ns	ns	ns
p biochar (B)	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values from the interaction slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01, and 0.001 probability level, respectively. $n = 3$: three replications per treatment.

Table 8. Average CH_4 emissions observed in treatments of the experiment (mean ± standard deviation).

-	Days of Experiment										
Treatments	Day 1	Day 2-3	Day 4-7	Day 8-18	Day 19-42	Day 43-74	Day 44-75	Day 76-121	Day 122-138	Day 139-195	∑0-195
-	(mg CH_4 m^{-2} day^{-1})										(kg CH_4 ha^{-1})
Control	-1 ± 1 b	1 ± 1 b	1 ± 1 a	1 ± 1 a	1 ± 1 a	2 ± 4 a	1 ± 1 a	-1 ± 1 ab	1 ± 1 a	1 ± 1 ab	3.0 ± 1.8 a
Biochar	-1 ± 1 b	-1 ± 1 b	1 ± 1 a	1 ± 1 a	1 ± 1 a	8 ± 2 a	2 ± 1 a	1 ± 1 ab	5 ± 1 a	3 ± 1 a	5.2 ± 1.5 a
WS	1 ± 1 ab	3 ± 1 a	2 ± 1 a	11 ± 9 a	2 ± 1 a	2 ± 5 a	-1 ± 1 a	1 ± 1 ab	1 ± 1 a	1 ± 1 b	7.8 ± 6.8 a
WS+Biochar	8 ± 7 a	3 ± 1 a	5 ± 1 a	9 ± 5 a	2 ± 1 a	7 ± 1 a	1 ± 1 a	1 ± 1 a	1 ± 1 a	1 ± 1 ab	13.8 ± 3.3 a
SF	-1 ± 1 b	1 ± 1 b	1 ± 1 a	1 ± 1 a	1 ± 1 a	7 ± 1 a	1 ± 1 a	1 ± 1 a	1 ± 1 a	1 ± 1 ab	7.1 ± 1.6 a
SF+Biochar	1 ± 1 ab	1 ± 1 b	1 ± 1 a	1 ± 1 a	1 ± 1 a	8 ± 2 a	1 ± 1 a	-1 ± 1 b	1 ± 1 a	1 ± 1 ab	4.4 ± 0.5 a
LF	1 ± 1 ab	1 ± 1 b	1 ± 1 a	1 ± 1 a	1 ± 1 a	10 ± 1 a	-1 ± 1 a	-1 ± 1 ab	1 ± 1 a	1 ± 1 ab	7.4 ± 2.3 a
LF+Biochar	-1 ± 1 b	1 ± 1 ab	1 ± 1 a	5 ± 4 a	1 ± 1 a	8 ± 1 a	1 ± 1 a	-1 ± 1 b	1 ± 1 a	1 ± 1 ab	7.2 ± 0.8 a
p slurries (A)	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
p biochar (B)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values from the interaction slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01, and 0.001 probability level, respectively. $n = 3$: three replications per treatment.

Table 9. Cumulative emissions and yields observed in treatments of the experiment (mean ± standard deviation).

-	GWP	Yield	Yield	Apparent N Recovery	N Use Efficiency	Yield-scaled GWP
Treatments	ton N ha^{-1}	ton DM ha^{-1}	kg N ha^{-1}	% N Applied	kg DM kg N-1	ton CO_2-eq. ton^{-1}
Control	19.2 ± 0.8 b	2.6 ± 0.1 e	25.9 ± 1.2 e	-	-	7.5 ± 0.2 ab
Biochar	19.0 ± 4.1 b	2.8 ± 0.1 e	35.7 ± 0.5 d	-	-	6.8 ± 1.5 ab
WS	16.6 ± 4.4 b	4.6 ± 0.2 b	79.7 ± 3.2 b	67.2 ± 4.0 a	26.0 ± 2.7 b	3.7 ± 1.1 b
WS+Biochar	26.5 ± 3.6 ab	4.4 ± 0.1 bc	86.7 ± 2.5 a	75.9 ± 3.1 a	23.3 ± 1.1 bc	5.9 ± 0.7 ab
SF	39.3 ± 3.6 a	5.9 ± 0.2 a	86.4 ± 2.3 a	75.6 ± 2.8 a	41.8 ± 2.2 a	6.7 ± 0.8 ab
SF+Biochar	29.3 ± 2.6 ab	5.6 ± 0.1 a	84.2 ± 2.4 ab	72.9 ± 3.0 a	37.3 ± 1.8 a	5.3 ± 0.6 ab
LF	20.2 ± 5.9 b	4.1 ± 0.1 cd	65.3 ± 0.9 c	49.2 ± 1.1 b	19.2 ± 0.8 cd	4.9 ± 1.4 ab
LF+Biochar	32.3 ± 9.6 ab	3.8 ± 0.1 d	60.8 ± 0.3 c	43.6 ± 0.4 b	15.3 ± 0.3 d	8.5 ± 2.5 a
p slurries (A)	ns	***	***	***	***	ns
p biochar (B)	ns	ns	ns	ns	*	ns
A × B	ns	ns	**	ns	ns	ns

Note: Values from the interaction slurries additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ns, *, ** and *** mean that the factor or interaction effects were not significant or significant at the 0.05, 0.01, and 0.001 probability level, respectively. $n = 3$: three replications per treatment.

3.3. Crop Productivity

The DM yield, expressed in DM per ha, in treatments that received slurries was significantly higher ($p < 0.05$) than in control, with increases between 60 and 130% (Table **9**). The DM yield, expressed in DM per ha, was not significantly different ($p > 0.05$) between treatments with and without biochar (Table **9**). The DM yield, expressed in N per ha, was significantly higher ($p < 0.05$) in treatments that received slurries than in Control, with increases in the following order: SF > WS > LF (Table **9**). The DM yield, expressed in N per ha, from the Biochar treatment was significantly higher ($p < 0.05$) than in Control (more than 38%), while the DM yield from WS+Biochar was higher ($p > 0.05$) than that of the WS treatment (Table **9**). The apparent N recovery in treatments receiving WS/SF was significantly higher ($p < 0.05$) than in the LF treatment (> 67.2% of N applied for WS/SF against 49.2% of N applied for LF) (Table **9**). No significant differences ($p > 0.05$) were observed in apparent N recovery between treatments with and without biochar (Table **9**). The N use efficiency increased significantly ($p < 0.05$) in treatments that received slurries in the following order: SF > WS > LF, with more than 41 kg DM kg^{-1} N for SF and from 19 to 26 kg DM kg^{-1} N for WS/LF (Table **9**). The N use efficiency was not significantly different ($p > 0.05$) between treatments with and without biochar, although a numerical reduction of 10 to 20% in biochar treatments was observed (Table **9**).

4. DISCUSSION

The application of slurries (WS, SF, and LF) to amended treatments increased the NH_4^+ concentration in the first two weeks due to the high NH_4^+: total N ratio (0.74 to 0.92) (Table **2**). Although the SF had significantly higher contents of DM and total C than the WS/LF, immobilization seems to have had no impact on the reduction of NH_4^+ availability in this slurry fraction. The lack of differences in NH_4^+ concentrations in treatments with and without biochar (Table **3**) was consistent with previous studies [14, 19], which reported that the application of biochar into the soil led to NH_4^+ ion adsorption, as biochar can act as a cation exchange medium and has a high capacity for N sorption. As can be observed in Table **2**, the characteristics of the three slurries were distinct, and the application rate was based on total N (80 kg N ha^{-1}). Hence, the total C applied in SF was significantly higher than in WS/LF, whereas NH_4^+ applied was significantly lower in SF. Previous studies [28, 29] observed that the high C/N ratio of the SF can induce a higher immobilization of N by the soil microbial biomass. The lack of differences in NO_3^- concentrations between SF and WS/LF without and with biochar could be related to the NO_3^- leaching by the high rainfall that occurred between October and February (855 mm) and represented 70% of the cumulative rainfall during the experiment (Table **1**). However, the addition of manure with biochar had the potential to decrease the N leaching losses by 11% when compared to manure only [30]. Saarnio *et al*. [31] reported that the application of biochar at rates of 1.0 to 3.0 kg m^{-2} does not contribute to the reduction of GHG emissions nor to the reduction of N or P leaching from peat soil in the short term, suggesting that larger quantities are needed. The application of slurries (WS, SL, and LF) led to the addition of significant amounts of N in organic and mineral forms to the soil. Some NH_4^+ can be nitrified, releasing H^+ that decreases soil pH [5], which is different from when the plant exists in the system [32, 33]. Similar studies have reported that the addition of biochar to soil increases overall pH because pyrolysis leads to the accumulation of alkaline substances on the biochar surface, which increases the soil pH [9, 11, 18, 19, 34-36].

The N_2O emissions came from the nitrification and denitrification processes, depending on the soil water content. Denitrification is the main source of N_2O fluxes from agricultural soil amended with slurries [37]. The increase in N_2O emissions from the treatments that received slurries in relation to the control is due to the addition of high concentrations of NH_4^+, organic N, and readily available organic C, increasing the processes of nitrification and denitrification (Table **2**) [38]. The availability of organic compounds as a C source in WS could be the reason for higher N_2O emissions from this treatment relative to SF/LF. Previous studies have reported that biochar application to soils could decrease, increase, or have no effect on N_2O, CO_2, and CH_4 emissions, depending on soil texture, biochar type, and their co-application with organic or inorganic fertilizers [16-19, 39, 40]. The following mechanisms are involved after biochar application into the soil: (i) reduction of NO_3^- to N_2, N_2O/N_2 ratio, and N_2O losses by the increase of soil pH [41]; (ii) reduction of denitrification and N_2O losses by the improvement of soil aeration [42]; and (iii) reduction of inorganic N availability and N_2O losses due N immobilization. The results of the present study put forward that to reach a significant N_2O reduction in soil with a sandy-loam texture, higher amounts (> 1.0 kg m^{-2}) of biochar are needed. In acidic or coarse-textured soils, previous studies [43, 44] reported an increase in crop yields with increasing biochar application rates (0.5-15.0 kg m^{-2}), which may be attributed to the liming effect and enhanced soil water storage, potentially improving nutrient availability. . Although results from different soil types cannot be directly extrapolated, the impact on soil N losses after biochar application depends on the physicochemical properties and modifications in the abundance and diversity of the microbial community [31, 45].

Carbon dioxide emissions are due to soil respiration, depending on the soil texture, water content, temperature, aeration, microbial activity and C mineralization, crop residues, and organic and inorganic fertilizer use [16, 29, 46, 47]. The application of slurries increases soil microbial activity and CO_2 fluxes due to the mineralization of organic matter, whereas rainfall reduces the availability of organic fertilizer [29]. In this study, the CO_2 emissions from the SF treatment were higher relative to WS/LF, which was consistent with the significantly higher amount of total C added by SF (53.5 g kg^{-1} in SF against less than 33.7 g kg^{-1} in WS/LF) (Table **2**). Previous studies are not unanimous about the influence of biochar in CO_2 released from the soil depending on soil properties, temperature, or fertilizer type [16, 38, 48]. In this study, biochar increased, but not significantly, the cumulative CO_2 emissions from WS/LF relative to SF. This may be related to increased rates of C mineralization in these treatments, either due to mineralization of the labile C added with the biochar or through increased mineralization of the soil organic matter [38].

Methane emissions are due to soil aeration, and positive or negative fluxes are a result of CH_4 production by anaerobic methanogenic organisms and CH_4 consumption by aerobic methanotrophic organisms [49, 50]. Previous studies [29, 51] reported that the slurry application into soil enhances CH_4 emissions for a few days due to the release of dissolved CH_4 during storage. Additionally, the rainfall events enhance the net CH_4 emission from methanogenesis in soil. In this study, the rainfall events should have enhanced the methanogenic activity in the soil, allowing it to act as a CH_4 source (Tables **1** and **8**). Applying biochar to soil increases CH_4 absorption because it improves CH_4 oxidation through soil aeration, decreasing this loss over time [15, 52, 53]. However, in the present study, the addition of biochar to soil did not reduce the CH_4 emissions from slurries,

although a numerical reduction in SF was observed. This decrease in CH_4 emissions in SF could be related to the reduction of anaerobic conditions by biochar addition to the soil [54]. In any case, the results of the present study are in line with previous studies [15-17, 38], which reported that animal manure and biochar generally have little effect on CH_4 flux from soils. The results of the present study are in line with those of other studies, in which biochar had no impact on yield-scaled GWP without the application of an N fertilizer (Table **9**) [16, 19].

Slurry separation makes it feasible to concentrate DM, organic N, and P in the SF, which can then be exported from the farm to regions with nutrient shortfalls or directed toward other portions of the farm [7]. The SF is very rich in recalcitrant C fractions such as lignin, hemicellulose, and lignocellulose, whereas the LF is rich in labile C fractions and contains the largest fraction of the NH_4^+ of the WS, being stored in the farm until used as an organic fertilizer [55]. The amount of slurry applied in each treatment was based on total N, and consequently, the amount of NH_4^+ applied varied between treatments, being lower in SF compared to WS/LF (Table **2**). The slurry was applied in October, and 70% of the rain recorded during the experiment fell until January (Tables **1** and **2**), increasing the leaching of NO_3^- during this period, except in SF, due to immobilization, as it should be the dominant process given the high C/N ratio and low water-soluble C in relation to total C [29]. The higher DM yield in SF compared to that of WS/LF may also be related to N immobilization, which can reduce NO_3^- leaching between October and May. The use of biochar can improve soil properties, which results in greater crop growth and productivity under normal conditions, as well as in soils that present abiotic stresses due to the presence of heavy metals, salt, or organic contaminants [53, 56]. In the present study, the addition of biochar to slurries had no effect on N use efficiency, concurring with previous studies, which reported that, in temperate climates, soils are often in good condition, characterized by a perfectly adjusted soil pH and high nutrient availability (Table **9**) [17, 20]. To validate and expand the results observed in this short period, it is essential to conduct extensive, long-term investigations in future research to understand discrepancies in emissions and discover the most effective practices (rate, depth, and frequency) for using biochar in agricultural soils [57].

CONCLUSION

Data from this study indicated that the mechanical separation of the WS generates an LF and an SF with two distinct compositions, with significantly higher contents of DM and total C in SF. The addition of biochar to these three slurries significantly increased the soil pH and seemed to have no impact on the other physicochemical characteristics of the soil. The cumulative N_2O and CH_4 emissions did not differ significantly between the three slurries, whereas CO_2 emissions and GWP were significantly higher in SF treatment. The emissions of N_2O, CO_2, CH_4, and GWP were not significantly different between treatments with and without biochar. The DM yield, expressed in N per ha, increased significantly in SF > WS > LF, while the addition of biochar significantly increased DM yield in WS. The apparent N recovery in WS/SF was significantly higher than in LF, but these three slurries, with and without biochar, did not differ significantly in apparent N recovery. N use efficiency increased significantly in SF > WS > LF, whereas no differences were observed among these three slurries with and without biochar.

Thus, it can be concluded that the addition of biochar combined with WS or SF/LF to sandy-loam soil appears to have no impact on GHG emissions and ryegrass yield during the autumn/winter season. Overall, these findings suggest that amounts higher than 1.0 kg m^{-2} of biochar, combined with SF, may need to be applied to soils to reduce GHG emissions and nitrate leaching, and to increase N use efficiency and crop yield. Future studies are recommended to explore different soil types, crop species, andenvironmental conditions to validate these findings.

AUTHORS' CONTRIBUTION

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on request from the corresponding author.

FUNDING

This work was supported by National Funds from FCT - Portuguese Foundation for Science and Technology, under the projects UIDB/00681/2020 (https://doi.org/10.54499/UIDB/00681/2020), UIDB/04033/2020 (https://doi.org/10.54499/UIDB/04033/2020), UIDB/50006/2020 and UIDP/50006/2020 (Fundação para a Ciência e Tecnologia), and the projects WASTECLEAN PROJ/IPV/ID&I/019 (Polytechnic Institute of Viseu), WASTE2VALUE PDR2020-1.0.1-FEADER-032314 (Ministério da Agricultura) and FEEDVALUE PRR-C05-i03-I-000242 (European Union).

CONFLICT OF INTEREST

Dr. José Pereira is on the Editorial Advisory Board of the journal TOASJ.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Scarlat N, Fahl F, Dallemand JF, Monforti F, Motola V. A spatial analysis of biogas potential from manure in Europe. Renew Sustain Energy Rev 2018; 94: 915-30. http://dx.doi.org/10.1016/j.rser.2018.06.035

[2] Bittman S, Dedina M, Howard CM, Oenema O, Sutton MA. Options for ammonia mitigation: guidance from the unece task force on reactive nitrogen. Project Reference: CEH Project no C04910. Edinburgh, UK: NERC/Centre for Ecology & Hydrology 2014; p. 83.

[3] Vastolo A, Serrapica F, Cavallini D, Fusaro I, Atzori AS, Todaro M. Editorial: Alternative and novel livestock feed: reducing environmental impact. Front Vet Sci 2024; 111441905 http://dx.doi.org/10.3389/fvets.2024.1441905 PMID: 39100757

[4] Sutton MA, Howard CM, Mason KE, Brownlie WJ. Cordovil CMdS Nitrogen Opportunities for Agriculture, Food & Environment. UNECE Guidance Document on Integrated Sustainable Nitrogen Management. Edinburgh, UK.: UK Centre for Ecology & Hydrology 2022; p. 157.

[5] IPCC Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Calvo Buendia E, Tanabe K, Kranjc A, Eds., *et al*. Geneva, Switzerland: IPCC 2019. Available from: http://www.ipcc-nggip.iges.or.jp (Accessed on: 16 August 2024).

[6] Fangueiro D, Surgy S, Fraga I, Cabral F, Coutinho J. Band application of treated cattle slurry as an alternative to slurry injection: Implications for gaseous emissions, soil quality, and plant growth. Agric Ecosyst Environ 2015; 211: 102-11. http://dx.doi.org/10.1016/j.agee.2015.06.003

[7] Prado J, Fangueiro D, Alvarenga P, Ribeiro H. Assessment of the agronomic value of manure-based fertilizers. Agronomy 2022; 13(1): 140. http://dx.doi.org/10.3390/agronomy13010140

[8] Bhattacharyya PN, Sandilya SP, Sarma B, *et al*. Biochar as soil amendment in climate-smart agriculture: opportunities, future prospects, and challenges. J Soil Sci Plant Nutr 2024; 24(1): 135-58. http://dx.doi.org/10.1007/s42729-024-01629-9

[9] Ebrahimi M, Souri MK, Mousavi A, Sahebani N. Biochar and vermicompost improve growth and physiological traits of eggplant (*Solanum melongena* L.) under deficit irrigation. Chem Biol Technol Agric 2021; 8(1): 19. a http://dx.doi.org/10.1186/s40538-021-00216-9

[10] Ebrahimi M, Mousavi A, Souri MK, Sahebani N. Can vermicompost and biochar control *Meloidogyne javanica* on eggplant? Nematology 2021; 23(9): 1053-64. b http://dx.doi.org/10.1163/15685411-bja10094

[11] Li B, Guo Y, Liang F, *et al*. Global integrative meta-analysis of the responses in soil organic carbon stock to biochar amendment. J Environ Manage 2024; 351119745 http://dx.doi.org/10.1016/j.jenvman.2023.119745 PMID: 38061094

[12] Paustian K, Lehmann J, Ogle S, Reay D, Robertson GP, Smith P. Climate-smart soils. Nature 2016; 532(7597): 49-57. http://dx.doi.org/10.1038/nature17174 PMID: 27078564

[13] Purakayastha TJ, Bera T, Bhaduri D, *et al*. A review on biochar modulated soil condition improvements and nutrient dynamics concerning crop yields: Pathways to climate change mitigation and global food security. Chemosphere 2019; 227: 345-65. http://dx.doi.org/10.1016/j.chemosphere.2019.03.170 PMID: 30999175

[14] Viaene J, Peiren N, Vandamme D, *et al*. Biochar amendment to cattle slurry reduces NH_3 emissions during storage without risk of higher NH_3 emissions after soil application of the solid fraction. Waste Manag 2023; 167: 39-45. http://dx.doi.org/10.1016/j.wasman.2023.05.023 PMID: 37244007

[15] Wang Y, Gu J, Ni J. Influence of biochar on soil air permeability and greenhouse gas emissions in vegetated soil: A review. Biogeotechnics 2023; 1(4)100040 http://dx.doi.org/10.1016/j.bgtech.2023.100040

[16] Abagandura GO, Chintala R, Sandhu SS, Kumar S, Schumacher TE. Effects of biochar and manure applications on soil carbon dioxide, methane, and nitrous oxide fluxes from two different soils. J Environ Qual 2019; 48(6): 1664-74. http://dx.doi.org/10.2134/jeq2018.10.0374

[17] Agegnehu G, Bass AM, Nelson PN, Bird MI. Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. Sci Total Environ 2016; 543(Pt A): 295-306. http://dx.doi.org/10.1016/j.scitotenv.2015.11.054 PMID: 26590867

[18] Bai SH, Omidvar N, Gallart M, *et al*. Combined effects of biochar and fertilizer applications on yield: A review and meta-analysis. Sci Total Environ 2022; 808152073 http://dx.doi.org/10.1016/j.scitotenv.2021.152073 PMID: 34863750

[19] Barracosa P, Cardoso I, Marques F, *et al*. Effect of biochar on emission of greenhouse gases and productivity of cardoon crop (*Cynara cardunculus* L.). J Soil Sci Plant Nutr 2020; 20(3): 1524-31. http://dx.doi.org/10.1007/s42729-020-00242-w

[20] Jaufmann E, Schmid H, Hülsbergen KJ. Effects of biochar in combination with cattle slurry and mineral nitrogen on crop yield and nitrogen use efficiency in a three-year field experiment. Eur J Agron 2024; 156127168 http://dx.doi.org/10.1016/j.eja.2024.127168

[21] Wang F, He Z, Zhang X, *et al*. Comparative effects of straw and biochar on N2O emissions from acidic soils. J Soil Sci Plant Nutr 2024; 24(2): 2080-90. http://dx.doi.org/10.1007/s42729-024-01742-9

[22] World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. 2015. Available from: http://www.fao.org/3/i3794en/I3794en.pdf

[23] Ovejero J, Ortiz C, Boixadera J, *et al*. Pig slurry fertilization in a double-annual cropping forage system under sub-humid Mediterranean conditions. Eur J Agron 2016; 81: 138-49. http://dx.doi.org/10.1016/j.eja.2016.09.005

[24] Regueiro I, Coutinho J, Gioelli F, Balsari P, Dinuccio E, Fangueiro D. Acidification of raw and co-digested pig slurries with alum before mechanical separation reduces gaseous emission during storage of solid and liquid fractions. Agric Ecosyst Environ 2016; 227: 42-51. http://dx.doi.org/10.1016/j.agee.2016.04.016

[25] Ferlizza E, Fasoli S, Cavallini D, Bolcato M, Andreani G, Isani G. Preliminary study on urine chemistry and protein profile in cows and heifers. Pak Vet J 2020; 40(4): 413-8. http://dx.doi.org/10.29261/pakvetj/2020.067

[26] Raspa F, Schiavone A, Pattono D, *et al*. Pet feeding habits and the microbiological contamination of dog food bowls: effect of feed type, cleaning method and bowl material. BMC Vet Res 2023; 19(1): 261. http://dx.doi.org/10.1186/s12917-023-03823-w PMID: 38062425

[27] Bordin C, Raspa F, Greppi M, *et al*. Pony feeding management: the role of morphology and hay feeding methods on intake rate, ingestive behaviors and mouth shaping. Front Vet Sci 2024; 111332207 http://dx.doi.org/10.3389/fvets.2024.1332207 PMID: 38681853

[28] Dosch P, Gutser R. Reducing N losses (NH3, N2o, N2) and immobilization from slurry through optimized application techniques. Fert Res 1996; 43(1-3): 165-71. http://dx.doi.org/10.1007/BF00747697

[29] Pereira J, Fangueiro D, Chadwick DR, Misselbrook TH, Coutinho J, Trindade H. Effect of cattle slurry pre-treatment by separation and addition of nitrification inhibitors on gaseous emissions and N dynamics: A laboratory study. Chemosphere 2010; 79(6): 620-7. http://dx.doi.org/10.1016/j.chemosphere.2010.02.029 PMID: 20202667

[30] Laird D, Fleming P, Wang B, Horton R, Karlen D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 2010; 158(3-4): 436-42. http://dx.doi.org/10.1016/j.geoderma.2010.05.012

[31] Saarnio S, Kekkonen H, Lång K. Addition of softwood biochar did not reduce N_2O emissions or N leaching from peat soil in the short term. Sci Total Environ 2024; 944173906 http://dx.doi.org/10.1016/j.scitotenv.2024.173906 PMID: 38871319

[32] Souri MK, Römheld V. Split daily applications of ammonium can not ameliorate ammonium toxicity in tomato plants. Hortic Environ Biotechnol 2009; 50: 384-91.

[33] Souri MK. Effectiveness of chloride compared to 3, 4-dimethylpyrazole phosphate on nitrification inhibition in soil. Commun Soil Sci Plant Anal 2010; 41(14): 1769-78. http://dx.doi.org/10.1080/00103624.2010.489139

[34] Ameloot N, Sleutel S, Das KC, Kanagaratnam J, de Neve S. Biochar amendment to soils with contrasting organic matter level: effects on N mineralization and biological soil properties. Glob Change Biol Bioenergy 2015; 7(1): 135-44. http://dx.doi.org/10.1111/gcbb.12119

[35] Kizito S, Luo H, Lu J, Bah H, Dong R, Wu S. Role of nutrient-enriched biochar as a soil amendment during maize growth: exploring practical alternatives to recycle agricultural residuals and to reduce chemical fertilizer demand. Sustainability 2019; 11(11): 3211. http://dx.doi.org/10.3390/su11113211

[36] Šimanský V, Klimaj A. How does biochar and biochar with nitrogen fertilization influence soil reaction? J Ecol Eng 2017; 18(5): 50-4. http://dx.doi.org/10.12911/22998993/74948

[37] Kool DM, Dolfing J, Wrage N, Van Groenigen JW. Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. Soil Biol Biochem 2011; 43(1): 174-8. http://dx.doi.org/10.1016/j.soilbio.2010.09.030

[38] Troy SM, Lawlor PG, O' Flynn CJ, Healy MG. Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. Soil Biol Biochem 2013; 60: 173-81. http://dx.doi.org/10.1016/j.soilbio.2013.01.019

[39] Spokas KA, Reicosky DC. Impacts of sixteen biochars in soil greenhouse gas production. Ann Environ Sci 2009; 3: 179-93.

[40] Zhang A, Liu Y, Pan G, *et al*. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant Soil 2012; 351(1-2): 263-75. http://dx.doi.org/10.1007/s11104-011-0957-x

[41] Verhoeven E, Six J. Biochar does not mitigate field-scale N2O emissions in a northern california vineyard: An assessment across two years. Agric Ecosyst Environ 2014; 191: 27-38. http://dx.doi.org/10.1016/j.agee.2014.03.008

[42] Ameloot N, Maenhout P, De Neve S, Sleutel S. Biochar-induced N2O emission reductions after field incorporation in a loam soil. Geoderma 2016; 267: 10-6. http://dx.doi.org/10.1016/j.geoderma.2015.12.016

[43] Castaldi S, Riondino M, Baronti S, *et al*. Impact of biochar application to a mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. Chemosphere 2011; 85(9): 1464-71. http://dx.doi.org/10.1016/j.chemosphere.2011.08.031 PMID: 21944041

[44] H DeLuca T, Thomas HDL. Influence of biochar on soil nutrient transformations, nutrient leaching, and crop yield. Adv Plants Agric Res 2016; 4(5): 348-62. http://dx.doi.org/10.15406/apar.2016.04.00150

[45] Clough T, Condron L, Kammann C, Müller C. A review of biochar and soil nitrogen dynamics. Agronomy 2013; 3(2): 275-93. http://dx.doi.org/10.3390/agronomy3020275

[46] Akinbi GO, Ngatia LW, Grace JM III, *et al*. Organic matter composition and thermal stability influence greenhouse gases production in subtropical peatland under different vegetation types. Heliyon 2022; 8(11)e11547 http://dx.doi.org/10.1016/j.heliyon.2022.e11547 PMID: 36406684

[47] Mon WW, Toma Y, Ueno H. Combined effects of rice husk biochar and organic manures on soil chemical properties and greenhouse gas emissions from two different paddy soils. Soil Syst 2024; 8(1): 32. http://dx.doi.org/10.3390/soilsystems8010032

[48] Fidel RB, Laird DA, Parkin TB. Effect of biochar on soil greenhouse gas emissions at the laboratory and field scales. Soil Syst 2019; 3(1): 8. http://dx.doi.org/10.3390/soilsystems3010008

[49] Abalos D, Sanchez-Martin L, Garcia-Torres L, van Groenigen JW, Vallejo A. Management of irrigation frequency and nitrogen fertilization to mitigate GHG and NO emissions from drip-fertigated crops. Sci Total Environ 2014; 490: 880-8. http://dx.doi.org/10.1016/j.scitotenv.2014.05.065 PMID: 24908647

[50] Li S, Chan CY. Will biochar suppress or stimulate greenhouse gas emissions in agricultural fields? unveiling the dice game through data syntheses. Soil Syst 2022; 6(4): 73. http://dx.doi.org/10.3390/soilsystems6040073

[51] Vallejo A, Meijide A, Boeckx P, *et al*. Nitrous oxide and methane emissions from a surface drip-irrigated system combined with fertilizer management. Eur J Soil Sci 2014; 65(3): 386-95. http://dx.doi.org/10.1111/ejss.12140

[52] Liu X, Mao P, Li L, Ma J. Impact of biochar application on yield-scaled greenhouse gas intensity: A meta-analysis. Sci Total Environ 2019; 656: 969-76. http://dx.doi.org/10.1016/j.scitotenv.2018.11.396 PMID: 30625683

[53] Murtaza G, Ditta A, Ullah N, Usman M, Ahmed Z. Biochar for the management of nutrient impoverished and metal contaminated soils: preparation, applications, and prospects. J Soil Sci Plant Nutr 2021; 21(3): 2191-213. http://dx.doi.org/10.1007/s42729-021-00514-z

[54] Rondon M, Romero M, Acevedo D, *et al*. Carbon sequestration potential of the neotropical savannas of Colombia and Venezuela. In: Lal R, Ceri C, Bernoux M, Etchevers J, Cerri E, Eds. Carbon Sequestration in Soils of Latin America. New York, USA: Food Products Press 2006.

[55] Fangueiro D, Merino P, Pantelopoulos A, Pereira JLS, Amon B, Chadwick DR. The Implications of Animal Manure Management on Ammonia and Greenhouse Gas Emissions. In: Bartzanas T, Ed. Technology for Environmentally Friendly Livestock Production Smart Animal Production. Cham: Springer 2023. http://dx.doi.org/10.1007/978-3-031-19730-7_5

[56] Gómez-Muñoz B, Case SDC, Jensen LS. Pig slurry acidification and separation techniques affect soil N and C turnover and N2O emissions from solid, liquid and biochar fractions. J Environ Manage 2016; 168: 236-44. http://dx.doi.org/10.1016/j.jenvman.2015.12.018 PMID: 26716355

[57] Shrestha RK, Jacinthe PA, Lal R, *et al*. Biochar as a negative emission technology: A synthesis of field research on greenhouse gas emissions. J Environ Qual 2023; 52(4): 769-98. http://dx.doi.org/10.1002/jeq2.20475 PMID: 36905388