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Effects of the Addition of Different Additives before Mechanical Separation of Pig Slurry on Composition and Gaseous Emissions

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Abstract: The treatment of animal slurry is used to improve management on a farm scale. The aim of this laboratory study was to assess the effects of the addition of the additives biochar, alum and clinoptilolite before the mechanical separation of whole pig slurry (WS) on the characteristics and emission of NH_3, N_2O, CO_2 and CH_4 from solid (SF) and liquid fractions (LF). The additives were mixed with WS (5% *w*/*w*), followed by separation, in a total of 12 treatments with 3 replicates, including the controls and WS with additives. Gaseous emissions were measured for 30 d by a photoacoustic multigas monitor, and initial characteristics of the slurries were assessed. The results indicated that the separation of the WS modified the initial physicochemical characteristics and increased the GWP emissions of the SF and LF, but not the NH_3 losses. However, the addition of additives before separation increased the nutrient value and reduced the GWP emissions from the SF and LF. Additionally, just the additive alum was effective in the reduction of *E. coli*. The additives led to significant reductions in NH_3 and N_2O emissions, with higher reductions in NH_3 losses for alum (51% for NH_3) and similar N_2O losses for all additives (70% for N_2O) observed, whereas the CO_2 and CH_4 emissions were reduced by biochar (25% for CO_2 and 50% for CH_4) and alum (33% for CO_2 and 30% for CH_4) but not by clinoptilolite. Although the additives had a positive effect on slurry management, it can be concluded that the addition of alum before mechanical separation has the potential to be the best mitigation measure because it improves the nutrient content and sanitation and decreases gaseous losses from slurry management.

Keywords: ammonia; greenhouse gases; mitigation measure; solid–liquid separation; slurry additives

1. Introduction

High livestock densities and the subsequent generation of large quantities of animal slurry (liquid manure) in certain areas of the world generate hotspots of increased environmental risks through ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4) and greenhouse gases emissions (nitrous oxide (N_2O)) [1]. The key solution to minimise or avoid environmental and health concerns associated with animal slurry is to ensure the appropriate management through the entire slurry chain from animal housing, storage, treatment operations and the application to soil [1,2].

Animals excrete most of the non-metabolised N as urea ($CO(NH_2)_2$), but birds excrete uric acid, which rapidly hydrolyses under the influence of the ubiquitous enzyme, urease, into ammonium (NH_4^+). Ammonium is in equilibrium with the NH_3 that is lost by volatilisation, also leading to CO_2 emissions by the dissociation of ammonium carbonate ($(NH_4)_2CO_3$) into NH_4^+, CO_2 and H_2O [3]. The hydrolysis reaction consumes H^+ with an increase in pH, consequently leading to an imbalance in the NH_4^+/NH_3 balance, increasing the volatilisation of NH_3 [3]. The solid fraction is more likely to be the source of CH_4 and CO_2 production by anaerobic decomposition of the organic matter, and to some extent, N_2O by nitrification and denitrification processes [2,3].

Previous studies [4–6] have compiled and fully described most of the Best Available Techniques (BAT) for mitigation of the environmental impacts (namely NH_3 and greenhouse gas (GHG) emissions) associated with animal slurry management. The technical report prepared by Foged et al. [7], the guidance document from the UNECE Task force on reactive nitrogen [8] and the UNECE Guidance document on sustainable nitrogen management [9] are also good summaries of BAT. Under the generic denomination of slurry additives is a group of products made up of different compounds that interact with the slurry, changing its chemical, biological and physical characteristics and properties [10]. The following positive effects are claimed and described to different degrees on the label of every product: a reduction in the emission of several gaseous compounds (NH_3 and H_2S); a reduction in unpleasant odours; a change in the physical properties of the manure to make it easier to handle; an increase in the fertilising value of the slurry; a stabilisation of pathogenic microorganisms. Several additives are marketed to reduce the NH_3 emission and odours from stored slurry but are not listed in the UNECE guidance document due to limited evidence of their efficacy and to clear independence during their testing process [8,10,11].

Mechanical separation of animal slurries on European farms, into a liquid and a relatively solid fraction, is often the first manure management step adopted on farms with nutrient excesses [12,13]. Slurry separation allows the concentration of dry matter, organic N and phosphorus (P) in the solid fraction, which can be used to target other parts of the farm where soil P status is suitable or exported from the farm to areas with nutrient deficiencies. The liquid fraction contains the largest fraction of the NH_4^+ and potassium (K) content of the original slurry and is often stored on the farm until used as an organic fertilizer in proximate soils [14]. The separation of the liquid fraction from dry matter reduces the requirement for expensive storage and improves the manageability of the liquid during pumping and soil application [15]. Furthermore, treatments of the slurry have been proposed to alter the chemical and physical characteristics of the separation influent, e.g., acidification, flocculation or coagulation to increase the efficiency of the separation treatment [16,17]. Thus, the addition of different additives before mechanical separation of pig slurry could modify the composition of the separated fractions and then further reduce gaseous losses.

The aim of this laboratory study was to assess, during short term storage, the effects of the addition of additives biochar, alum and clinoptilolite before the mechanical separation of pig slurry on the characteristics and emission of NH_3, N_2O, CO_2 and CH_4 from the resulting solid and liquid fractions.

2. Materials and Methods

2.1. Slurries and Additives

Whole pig slurry was obtained via the intensive fattening of a pig from a commercial farm located in Viseu, Portugal. Each one of the additives biochar, (Bioc), alum (Alum) and clinoptilolite (Clin), were mixed into a sample (20 kg) of whole slurry (WS) at a rate of 5% (*w*/*w*), using closed plastic barrels at 20 °C for 24 h. Another sample (20 kg) of whole slurry (WS) without any additive was retained in a similar barrel and storage conditions. Then, 4 kg of WS with each one of the three additives (WS + Bioc, WS + Alum, and WS + Clin), as well as the same amount of WS without additive (WS), were kept in closed plastic barrels and subsamples were retained for analysis, before the start of the experiment. Additionally, the remaining samples (16 kg) of each one of the four slurries with and without additives were subjected to sieving through a 1.0 mm screen, generating a solid (SF) and a liquid fraction (LF), with the following separation yields (w/w): 26.3% for SF and 73.7% for LF; 28.4% for SF + Bio and 71.6% for LF + Bio; 29.7% for SF + Alum and 70.3% for LF + Alum; 29.2% for SF + Clin and 70.8% for LF + Clin. The sieving of WS through a 1.0 mm screen was to mimic the commercially mechanical separators used on commercial farms.

Samples of the WS with and without additives and their corresponding fractions, for a total of 12 treatments (with three replications per treatment), were subdivided into individual doses (1.0 kg) using plastic containers, and immediately frozen (−18 °C) until required for the laboratory experiments. Subsamples were retained and analysed by standard laboratory methods [18–20] to the physicochemical and biological properties shown in Table 1. Briefly, pH (H_2O) was determinate by potentiometry (EN 13037, Brussels, Belgium), dry matter content by the gravimetric method (EN 13040, Brussels, Belgium), total C by the Dumas method, total N by the Kjeldahl method (EN 13654-1, Brussels, Belgium), NH_4^+ and NO_3^- by absorption spectrophotometry (EN 13652, Brussels, Belgium) and *Escherichia coli* (*E. coli*) by colony count technique at 44 °C (ISO 16649-2, Geneva, Switzerland).

Table 1. Physicochemical and biological characteristics of the treatments at the beginning of the experiment (mean ± standard deviation) (n = 3).

Treatments	**pH**	**DM**	**TC**	**TN**	**NH_4^+**	**NO_3^-**	**NH_4^+/TN**	**C/N**	***E. coli***
WS	7.1 ± 0.1 bc	22.6 ± 3.0 e	43.8 ± 1.1 gh	3.3 ± 0.1 bcd	1.9 ± 0.1 cde	6 ± 1 a	0.6 ± 0.1 bc	13 ± 1 fg	244 ± 31 ab
WS + Bioc	7.1 ± 0.1 bc	84.5 ±0.4 c	55.0 ± 0.1 e	3.0 ± 0.1 bcd	1.9 ± 0.1 cde	4 ± 1 ab	0.6 ± 0.1 ab	18 ± 1 bcd	261 ± 28 ab
WS + Alum	3.9 ± 0.1 d	68.2 ± 0.7 cd	34.7 ± 0.6 i	3.3 ± 0.1 bcd	2.1 ± 0.1 bc	5 ± 1 a	0.6 ± 0.1 ab	11 ± 1 g	1 ± 1 c
WS + Clin	7.1 ± 0.1 bc	64.1 ± 3.9 cd	40.8 ± 0.6 h	2.6 ± 0.1 d	2.1 ± 0.1 bcd	5 ± 1 a	0.8 ± 0.1 a	16 ± 1 cdef	194 ± 13 ab
SF	7.0 ± 0.1 bc	137.7 ± 2.3 b	149.9 ± 0.4 a	6.8 ± 0.1 a	3.3 ± 0.1 a	5 ± 1 a	0.5 ± 0.1 bcd	22 ± 1 ab	252 ± 1 ab
SF + Bioc	7.1 ±0.1 bc	142.3 ± 1.0 b	152.9 ± 0.7 a	6.6 ± 0.2 a	3.0 ± 0.1 a	4 ± 1 ab	0.4 ± 0.1 cd	23 ± 1 a	348 ± 26 a
SF + Alum	4.2 ± 0.1 d	151.9 ± 1.4 ab	141.5 ± 0.6 b	6.5 ± 0.1 a	2.4 ± 0.1 b	2 ± 1 c	0.4 ± 0.1 d	22 ± 1 ab	1 ± 1 c
SF + Clin	6.9 ± 0.1 c	171.3 ± 11.5 a	114.3 ± 2.3 c	6.5 ± 0.1 a	2.3 ± 0.1 bc	1 ± 1 c	0.3 ± 0.1 d	18 ± cde	124 ± 24 bc
LF	7.7 ± 0.1 a	15.4 ± 0.3 e	53.8 ± 0.1 ef	3.7 ± 0.2 b	1.6 ± 0.1 def	2 ± 1 bc	0.4 ± 0.1 cd	15 ± 1 def	321 ± 31 a
LF + Bioc	7.4 ± 0.1 ab	65.0 ± 0.8 cd	62.5 ± 0.1 d	3.2 ± 0.1 bcd	1.6 ± 0.2 ef	2 ± 1 c	0.5 ± 0.1 bcd	19 ± 1 abc	332 ± 41 a
LF + Alum	4.0 ± 0.1 d	52.0 ± 0.4 d	48.2 ± 1.2 fg	3.5 ± 0.2 bc	1.3 ± 0.1 f	1 ± 1 c	0.4 ± 0.1 d	14 ± 1 efg	1 ± 1 c
LF + Clin	7.4 ± 0.1 abc	64.1 ± 0.1 cd	50.8 ± 1.1 ef	2.8 ± 0.2 cd	1.3 ± 0.1 f	1 ± 1 c	0.5 ± 0.1 cd	18 ± 1 bcd	236 ± 72 ab
p slurries (A)	***	***	***	***	***	***	***	***	ns
p additives (B)	***	***	***	***	***	***	*	***	*
A × B	**	***	***	ns	***	***	**	***	ns

Note: n = 3: three replications per treatment. pH: pH (H_2O), Dry matter: DM (g kg^{-1}), Total C: TC (g kg^{-1}), Total N: TN (g kg^{-1}), NH_4^+: NH_4^+-N (g N kg^{-1}), NO_3^-: NO_3^--N (mg N kg^{-1}), NH_4^+/TN: NH_4^+: total N ratio, C/N: C:N ratio, *Escherichia coli*: *E. coli* (colony-forming units (CFU) mL^{-1}). Data expressed on a fresh weight basis. 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, respectively, not significant or significant at the 0.05, 0.01 and 0.001 probability level.

The biochar was produced from wood shavings (Ø = 2 mm) after being pyrolyzed in a muffle furnace (900 °C) (Piroeco Bioenergy, S.L., Spain) with the following characteristics: particle size distribution of 552 g kg^{-1} for Ø > 0.30 mm, 364 g kg^{-1} for Ø = 0.20–0.30 mm, 41 g kg^{-1} for Ø = 0.15–0.20 mm and 43 g kg^{-1} for Ø > 0.15 mm (by sieving method); bulk density of 0.1219 g cm^{-3} (by core method); pH (H_2O) of 10.2 (by potentiometry); moisture content 102.4 g kg^{-1} (by gravimetric method); total C of 806.0 g kg^{-1} (by Dumas method); total N of 1.9 g kg^{-1} (by Kjeldahl method). The alum (aluminum sulfate hexadecahydrate ≥95%, p.a., cryst; Al_2 $(SO_4)_3$ * 16 H_2O) was supplied by Panreac (AppliChem GmbH, Darmstadt, Germany) with the following characteristics: CAS number of 16828-11-8; molecular weight of 630.4 g mol^{-1}; particle size of 0.4 mm; pH (H_2O) of 3.5. The sedimentary origin of the clinoptilolite was Turkey (Zeolita Natural NUTRI-Clinoptilolita 1g568, ZeoCat Soluciones Ecológicas S.L.U., Barcelona, Spain), with the following characteristics: particle size < 0.425 mm; mineralogical composition of 915 g kg^{-1} for clinoptilolite, 35 g kg^{-1} for montmorillonite, 35 g kg^{-1} for feldspars, 15 g kg^{-1} for muscovite and 10 g kg^{-1} for cristobalite (by XRD analysis); chemical composition of 685 g kg^{-1} for SiO_2, 110 g kg^{-1}

for Al_2O_3, 29 g kg^{-1} for K_2O, 31 g kg^{-1} for CaO, 11 g kg^{-1} for MgO, 4.5 g kg^{-1} for Na_2O and 0.5 g kg^{-1} for TiO_2; cation exchange capacity (CEC) of 1.7 meq g^{-1} (by CEC method); apparent porosity of 47.5%; specific surface area of 75 m^2 g^{-1} (by Brunauer, Emmett and Teller method); pH (H_2O) of 7.8; bulk density of powder of 750 kg m^{-3}; moisture content of 65.7 g kg^{-1}. The rate of each additive was chosen considering previous studies [20,21], which recommended a maximum dosage of 5% (w/w) due to economic and practical issues.

2.2. Gaseous Emissions

The experiment was carried out using a system of twelve Kilner jars (H = 230 mm, Ø = 105 mm, volume = 2.0 L) filled with 1.0 kg (H = 105 mm) of each treatment (with three replications per treatment) along 30 d and at constant airflow rate and temperature (20 °C), such as those used by Pereira et al. [21,22]. Briefly, one air inlet and one air outlet were inserted in the jar lid with a Teflon tube (Ø = 3 mm) through one of the septa, with the end kept above the slurry surface (H = 20 mm). The airflow through the headspace of each jar was achieved by a pump (KNF, model N010.KN.18, Neuberger GmbH, Freiburg, Germany), with a flowrate (2.5 L min^{-1}) regulated by a needle valve coupled to a flow meter (AalborgTM FT10201SAVN, Aalborg, Denmark). The inlet air was subjected to NH_3-trapping filters with oxalic acid and the outlet air of the Kilner jars was exhausted out of the climatic room by a fume hood. The concentrations of NH_3, N_2O, CO_2 and CH_4 were measured in the exhaust air with a photoacoustic multigas monitor (INNOVA 1412i-5, Lumasense Technologies, Ballerup, Denmark) and air samples collected, in sequence (2 min intervals), through one sampling point (Ø = 3 mm) per Kilner jar, by a multipoint sampler (INNOVA 1409-12, Lumasense Technologies, Ballerup, Denmark) provided with PTFE-filters (0.001 mm pore size, Whatman, Ome, Japan). The photoacoustic multigas monitor was equipped with an optical filter for water vapour (filter type SB0527) and the detection limits for NH_3 (filter type UA0973), N_2O (filter type UA0985), CO_2 (filter type UA0982) and CH_4 (filter type UA0969) were, respectively, 0.1521, 0.0589, 2.9471 and 0.2864 mg m^{-3}. The photoacoustic multigas monitor was calibrated by the manufacturer before the beginning of the experiment and operated in a mode that compensated for water interference and cross interference.

For each experiment, individual samples of WS with and without additives and their corresponding fractions were thawed (over 24 h at 4 °C) and then brought to 20 °C immediately before being inserted in the Kilner jar. The temperature was monitored without interruption by sensors (CS107, Campbell Scientific, Loughborough, UK) connected to a micrologger (CR3000, Campbell Scientific, Loughborough, UK).

2.3. Data Analysis

The NH_3, N_2O, CO_2 and CH_4 concentrations were used to determinate means per hour and day per each outlet sampling point. Then, the emission of these four gases was determined (per hour) using a mass balance as described in Equation (1):

$$E = F \times \left(\frac{OUT - IN}{A}\right) \quad (1)$$

where E is the gas emission (mg m^{-2} h^{-1}), F is the air flowrate in the Kilner jar (m^3 h^{-1}), OUT is the outlet gas concentration (mg m^{-3}), IN is the inlet gas concentration (mg m^{-3}) using the following background coefficients: 0.00266 mg m^{-3} for NH_3, 0.58942 mg m^{-3} for N_2O, 628.71429 mg m^{-3} for CO_2 and 1.07411 mg m^{-3} for CH_4, and A (m^2) is the emitting surface area of the Kilner jar.

The reduction efficiencies (RE, %) of NH_3, CO_2, CH_4 and N_2O emissions from slurries and their fractions with additives, comparatively to untreated slurries, were determined as described in Regueiro et al. [17] using the Equation (2):

$$RE = 100 - ((AD/UN) \times 100) \quad (2)$$

where AD is the mean value of individual or cumulative gas values from slurries and their fractions with additives, and UN is the mean value of individual or cumulative gas values from untreated slurries.

The cumulative emissions of NH_3, N_2O, CO_2 and CH_4 were determined by averaging the flux between two sampling occasions and multiplying by the time interval between the measurements [21,22]. Then, cumulative emissions were expressed as % of total N or C applied in each slurry and fraction. The global warming potential (GWP) for each Kilner jar was assessed using the global warming potential coefficients for direct greenhouse gas emissions (265 for N_2O, 1 for CO_2 and 28 for CH_4) and indirect N_2O emissions (1% of NH_3-N volatilised for N_2O-N) [21–23].

To assess the effect of the separation process on gaseous emissions, the sum of the corrected cumulative emissions from slurries and their fractions with additives were compared with their respective unseparated slurries. The sum of the emissions was calculated by Equation (3):

$$\mathrm{GS} = (\mathrm{GLF} \times \mathrm{ALF}) + (\mathrm{GSF} \times \mathrm{ASF}) \tag{3}$$

where GS is the sum of the corrected cumulative emissions from separated slurries, GLF and GSF are the corrected cumulative emissions for liquid and solid fractions, respectively, and ALF and ASF are the proportions of liquid and solid fractions, respectively, obtained after the separation of additive and non-additive slurries.

The data obtained was analysed by two-way analysis of variance (ANOVA) to test the effects of dependent parameters (composition and gaseous emissions of slurries and their fractions with and without additives), followed by Tukey's significant difference test ($p < 0.05$) comparisons of means tests (for the factor (slurries or additives) or interaction effects), using the statistical software package STATISTIX 10.0 (Analytical Software, Tallahassee, FL, USA).

3. Results and Discussion

3.1. Composition of the Slurries

At the beginning (0 d) of the study, the main characteristics of the treatments that received slurries (WS, SF and LF) with and without additives (Bioc, Alum and Clin) are provided in Table 1. The initial pH values (0 d) did not differ significantly ($p > 0.05$) among treatments WS and SF (pH = 7.1), being significantly higher ($p < 0.05$) in treatment LF (pH = 7.7) (Table 1). In addition, the initial pH values of slurry treatments with the additive Alum (pH < 4.2) decreased significantly ($p < 0.05$) when compared with all other treatments (pH > 6.9) (Table 1). The initial dry matter content (0 d) did not differ significantly ($p > 0.05$) among treatments WS and LF (DM < 2.3%), being significantly higher ($p < 0.05$) in treatment SF (DM = 13.8%) (Table 1). The initial DM content increased significantly ($p < 0.05$) in almost all additive treatments (Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF) (2.3% for WS against 8.5% for WS + Bioc), with higher values for treatments WS and LF with additive Bioc (Table 1).

The initial total C (0 d) was significantly higher ($p < 0.05$) in treatment SF relative to treatments WS and LF (149.9 vs. 48.8 g total C kg^{-1}) (Table 1). The initial total C increased significantly ($p < 0.05$) in treatments WS and LF with the additive Bioc when compared with all other treatments. (Table 1). The initial total N (0 d) was significantly higher ($p < 0.05$) in treatment SF relative to treatments WS and LF (6.8 vs. 3.3 g total N kg^{-1}) (Table 1). The initial total N did not increase significantly ($p > 0.05$) in all additive treatments (Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF) (Table 1). The initial NH_4^+ (0 d) was significantly higher ($p < 0.05$) in treatment SF relative to treatments WS and LF (3.3 vs. 1.8 g total N kg^{-1}) (Table 1). The initial NH_4^+ did not increase significantly ($p > 0.05$) in all additive treatments (Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF) (Table 1). The initial NO_3^- (0 d) was significantly higher ($p < 0.05$) in treatments WS and SF relative to treatment LF (Table 1). The initial NO_3^- did not increase significantly ($p > 0.05$) in all additive treatments

(Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF) (Table 1). The initial NH_4^+/total N ratio (0 d) did not differ significantly ($p > 0.05$) among treatments WS, SF and LF (Table 1). The initial NH_4^+/total N ratio did not increase significantly ($p > 0.05$) in all additive treatments (Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF) (Table 1). The initial C/N ratio was significantly higher ($p < 0.05$) in treatment SF relative to treatments WS and LF (C/N = 22 for LF against C/N = 14 for WS or LF) (Table 1). The initial C/N ratio (0 d) did not increase significantly ($p > 0.05$) in almost all additive treatments (Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF) (Table 1).

The separation yields of the SF increased significantly ($p < 0.05$) in all slurries with additives (Bioc, Alum and Clin) relative to WS, with higher values for Alum (approximately 30%), in agreement with previous studies that reported an enhancement of separation yields due the addition of additives before the mechanical separation [12,14,16,17].

The additives interact with the whole slurry, changing its chemical, biological and physical characteristics and properties, with the following positive effects: reduction in the emission of several gaseous compounds, change in the physical properties of the manure to make it easier to handle, increase in the fertilising value of the manure and stabilisation of pathogenic microorganisms [3,8–10]. Biochar is a porous carbonaceous material largely containing C jointly with the inorganic components of the biomass utilised, such as alkali and alkaline earth metals, and its addition to slurry before separation increases the pH, the C/N ratio, cation-exchange capacity and microbial activities [22]. Clinoptilolite are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations with high porosity, ion exchange and adsorption capacity for NH_4^+ retention, and its addition to slurry before separation reduces the dissolved NH_4^+ by adsorbing on ion exchange sites [24]. Alum acts by acidification of the slurry at $pH < 5.0$, conserving NH_3, and its addition to slurry before separation improves fertilizer value and sanitisation [25].

The initial *E. coli* (0 d) did not differ significantly ($p > 0.05$) among treatments WS, SF and LF (Table 1). In addition, the initial *E. coli* of slurry treatments with the additive Alum decreased significantly ($p < 0.05$) when compared with all other additive treatments (Bioc and Clin) (1.0 colony-forming units (CFU) mL^{-1} for Alum) (Table 1). Results of this study (Table 1) did not show evidence that the addition of biochar and clinoptilolite might be effective at reducing *E. coli*, corroborating with the literature concerning the reduced effectiveness of these additives on bacterial activity [20,22]. On other hand, the addition of alum was effective on the reduction in *E. coli* (Table 1), being in line with previous studies where acidification was able to achieve sanitisation to $pH < 5.0$ [25].

3.2. Nitrogen Emissions

On most measurement days, the daily NH_3 fluxes decreased progressively in treatments throughout the 30 d of experiment (from 980 to 30 mg m^{-2} h^{-1}) and are shown in Table 2. Additionally, on day 30 of the experiment, significantly higher ($p < 0.05$) NH_3 fluxes were observed in the following order: LF > WS > SF, (Table 2). Comparative to the WS treatment, the SF treatment significantly reduced ($p < 0.05$) the daily NH_3 fluxes by 54% whereas the LF treatment increased these fluxes by 54% (Table 2). During most measurement days, the daily NH_3 fluxes were significantly reduced ($p < 0.05$) in all additive treatments (Bioc, Alum and Clin) when compared with the same treatments without additives (WS, SF and LF), with reductions of 50% for additives Alum and Clin and of 38% for additive Bioc (Table 2). The cumulative NH_3 emissions, expressed in g m^{-2}, increased significantly ($p < 0.05$) in the following order: LF > WS > SF, with a reduction of 53% for the SF treatment and an increase of 45% for the LF treatment when compared with the WS treatment (Table 2). The cumulative NH_3 emissions, expressed in g m^{-2}, were significantly reduced ($p < 0.05$) in all additive treatments (Bioc, Alum and Clin) relative to the same treatments without additives (WS, SF, LF), with reductions of 52% for the additives Alum and Clin and of 38% for the additive Bioc (Table 2). The cumulative NH_3 emissions, expressed as % of total N applied, increased significantly ($p < 0.05$) in the following order:

LF > WS > SF, with a reduction of 78% for the SF treatment and an increase of 28% for the LF treatment when compared with the WS treatment (Table 3). The cumulative NH_3 emissions, expressed as % of total N applied, were significantly reduced ($p < 0.05$) in all additive treatments (Bioc, Alum and Clin) relative to the same treatments without additives (WS, SF and LF), with reductions of 51% for the additive Alum and of 36% for the additives Bioc and Clin (Table 3).

As can be seen in Table 3, the NH_3 emissions were reduced by 36% by the addition of biochar or clinoptilolite and by 51% by the addition of alum, which could be related with saturation of the capacity of adsorption of NH_4^+ by biochar or clinoptilolite and the maintenance of low and stable pH by alum [24,26]. The reduction of NH_3 losses by biochar were due to the high specific surface area and the high cation exchange capacity of these additives, which enhance the NH_4^+ and NH_3 binding [26]. Previous studies [22,27] reported that the addition of biochar (1–12% w/w) to animal manure reduced NH_3 emissions between 12 and 77%, in the same range than the present study (36% NH_3 reduction for 5% w/w biochar). The addition of clinoptilolite increases the number of NH_4^+ exchange sites, decreasing the quantity of dissolved NH_4^+ and, thus, the quantity of equilibrated NH_3 gas available for NH_3 volatilisation [24]. In this study, the reduction of NH_3 emissions (36% NH_3 reduction for 5% w/w of clinoptilolite) by the addition of clinoptilolite was in line with emissions (26–50% NH_3 reduction for 2.50–6.25% w/w of clinoptilolite) reported in other studies [22,24] for animal slurry. The addition of alum was effective in conserving NH_3 because the percentage of total solution ammoniacal N (NH_4^+ and NH_3) that was dissociated as NH_3 gas is approximately 0.006% at a pH of 5.0 and temperature of 25 °C [24]. Previous studies [17,24] reported that the addition of alum (2.0–2.5% w/w) to animal slurry reduced NH_3 emissions by between 60 and 67%, being comparable with emissions of the present study (51% NH_3 reduction for 5% w/w of alum).

The daily N_2O fluxes follow the same trend in treatments, with a small variation throughout the 30 d of experiment, except in SF treatments with and without additives with a progressive increase in the last 20 d of the experiment (Table 4). Comparative to other treatment slurries, the daily N_2O fluxes were significantly higher ($p < 0.05$) in the first 11 d of the experiment for the LF treatment, and from day 16 until the end of the experiment for the SF treatment (Table 4). Comparative to treatments without additives, the daily N_2O fluxes were significantly reduced ($p < 0.05$) in the first 11 d of the experiment for the WS and SF treatments, and from day 12 until the end of the experiment for the SF treatment (Table 4). The cumulative N_2O emissions, expressed in g m^{-2}, were not significantly different ($p > 0.05$) among the WS and LF treatments, being lower by approximately 70% than the SF treatment (Table 4). The cumulative N_2O emissions, expressed in g m^{-2}, did not differ significantly ($p > 0.05$) among the WS and LF treatments with and without additives (Bioc, Alum and Clin), whereas these treatment additives were significantly reduced ($p < 0.05$) by 69% when compared with the SF treatment without additive (Table 4). The cumulative N_2O emissions, expressed as % of total N applied, did not differ significantly ($p > 0.05$) between the WS and LF treatments, but were significantly lower ($p < 0.05$) relative to the SF treatment (1.2% of total N applied for WS or LF treatments and 2.3% of total N applied for the SF treatment) (Table 3). The cumulative N_2O emissions, expressed as % of total N applied, were significantly reduced ($p < 0.05$) by approximately 70% in SF treatments with additives (Bioc, Alum and Clin) when compared with the same treatment without additive, whereas no significant reductions ($p > 0.05$) were observed in the WS or LF treatments with additives (Table 3).

Table 2. Average ammonia fluxes (mg m^{-2} h^{-1}) and total flux (mg m^{-2}) from each treatment (mean ± standard deviation) (n = 3).

Treatments	Days of Experiment										Total Flux
	1	**2**	**3–4**	**5–6**	**7–8**	**9–11**	**12–15**	**16–20**	**21–25**	**26–30**	**∑0–30**
WS	714 ± 7 b	573 ± 12 b	400 ± 9 c	358 ± 4 c	337 ± 2 c	353 ± 7 c	371 ± 3 b	290 ± 7 a	225 ± 3 a	183 ± 1 b	248,079 ± 1748 b
WS + Bioc	310 ± 51 e	322 ± 34 c	223 ± 1 d	226 ± 5 de	226 ± 1 de	235 ± 1 d	244 ± 2 d	175 ± 9 ef	136 ± 5 c	106 ± 5 d	148,694 ± 952 d
WS + Alum	170 ± 8 f	170 ± 8 de	98 ± 12 e	93 ± 14 fg	123 ± 6 fg	153 ± 5 ef	190 ± 2 e	166 ± 2 ef	150 ± 1 bc	147 ± 5 c	113,810 ± 3555 e
WS + Clin	338 ± 15 de	237 ± 3 cd	96 ± 10 e	81 ± 15 g	102 ± 12 fg	150 ± 4 ef	192 ± 3 e	199 ± 5 d	162 ± 3 b	128 ± 2 cd	123,814 ± 3098 e
SF	321 ± 14 de	244 ± 9 cd	196 ± 7 d	171 ± 5 ef	167 ± 5 ef	194 ± 8 de	215 ± 4 e	158 ± 3 f	80 ± 1 d	36 ± 1 ef	116,126 ± 2346 e
SF + Bioc	179 ± 8 f	136 ± 13 de	109 ± 14 e	105 ± 19 fg	91 ± 13 fg	100 ± 7 fg	125 ± 6 f	99 ± 12 g	51 ± 3 e	28 ± 3 f	68,082 ± 5807 f
SF + Alum	120 ± 1 f	89 ± 1 e	85 ± 1 e	81 ± 2 g	71 ± 1 g	67 ± 1 g	70 ± 1 g	70 ± 1 h	67 ± 1 de	52 ± 1 e	54,287 ± 67 f
SF + Clin	140 ± 2 f	101 ± 6 e	80 ± 9 e	71 ± 12 g	67 ± 13 g	80 ± 12 g	110 ± 8 f	92 ± 11 gh	58 ± 13 e	33 ± 4 ef	58,780 ± 7157 f
LF	978 ± 15 a	918 ± 27 a	924 ± 19 a	798 ± 10 a	670 ± 4 a	574 ± 3 a	442 ± 3 a	266 ± 3 b	225 ± 1 a	214 ± 1 a	359,038 ± 2693 a
LF + Bioc	598 ± 15 c	623 ± 51 b	662 ± 43 b	531 ± 38 b	455 ± 48 b	419 ± 35 b	267 ± 14 d	174 ± 4 ef	168 ± 3 b	179 ± 1 b	246,348 ± 9162 b
LF + Alum	299 ± 2 e	282 ± 3 c	280 ± 6 d	298 ± 2 cd	304 ± 5 cd	349 ± 5 c	252 ± 2 d	184 ± 6 de	161 ± 1 b	173 ± 10 b	178,865 ± 2980 c
LF + Clin	403 ± 7 d	231 ± 17 cd	199 ±15 d	230 ± 2 de	312 ± 5 c	357 ± 4 c	339 ± 1 c	222 ± 5 c	137 ± 3 c	133 ± 7 c	180,411 ± 672 c
p slurries (A)	***	***	***	***	***	***	***	***	***	***	***
p additives (B)	***	***	***	***	***	***	***	***	***	***	***
A × B	***	***	***	***	***	**	***	***	***	***	***

Note: n = 3: three replications per treatment. Values from the interaction slurries × additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ** and *** mean that the factor or interaction effects were, respectively, significant at the 0.01 and 0.001 probability level.

Table 3. Cumulative nitrogen and carbon emissions from each treatment (mean ± standard deviation) (n = 3).

Treatments	NH$_3$ (% Total N Applied)	N$_2$O (% Total N Applied)	N (g m^{-2})	N (% Total N Applied)	CO$_2$ (% Total C Applied)	CH$_4$ (% Total C Applied)	C (g m^{-2})	C (% Total C Applied)	GWP (g CO$_2$-eq. m^{-2})
WS	54.0 ± 5.0 b	1.2 ± 0.1 bcd	209 ± 25 b	55.2 ± 5.1 b	80.5 ± 4.3 a	3.3 ± 0.2 ab	4244 ± 11 c	83.8 ± 4.5 a	23,870 ± 127 c
WS + Bioc	34.9 ± 3.2 d	1.0 ± 0.1 cde	126 ± 2 d	35.9 ± 3.3 d	58.1 ± 0.9 cd	1.6 ± 0.3 d	3794 ± 89 cde	59.7 ± 1.3 cd	19,343 ± 1164 ef
WS + Alum	24.6 ± 0.8 e	1.0 ± 0.1 cde	98 ± 5 e	25.6 ± 0.8 e	86.8 ± 3.0 a	2.7 ± 0.5 bc	3588 ± 18 ef	89.5 ± 3.2 a	18,947 ± 762 ef
WS + Clin	33.7 ± 1.1 de	1.2 ± 0.1 bc	106 ± 4 e	34.9 ± 1.1 de	82.8 ± 4.1 a	3.7 ± 0.1 a	4074 ± 12 cd	86.5 ± 4.2 a	23,174 ± 326 cd
SF	12.1 ± 0.6 f	2.3 ± 0.1 a	114 ± 3 de	14.4 ± 0.6 f	31.8 ± 0.3 ef	1.7 ± 0.2 d	5790 ± 12 a	33.4 ± 0.1 e	39,893 ± 1495 a
SF + Bioc	7.3 ± 1.6 g	0.8 ± 0.1 e	63 ± 8 f	8.2 ± 1.7 g	23.4 ± 1.8 fg	0.5 ± 0.1 e	4235 ± 321 c	24.0 ± 2.0 f	21,884 ± 2018 cd
SF + Alum	5.9 ± 0.1 g	0.5 ± 0.1 f	48 ± 1 f	6.4 ± 0.2 g	21.3 ± 0.4 g	0.5 ± 0.1 e	3561 ± 24 f	21.8 ± 0.3 f	17,718 ± 254 f
SF + Clin	6.4 ± 1.3 g	0.9 ± 0.2 de	55 ± 11 f	7.4 ± 1.4 g	37.2 ± 2.0 e	1.4 ± 0.1 d	5104 ± 216 b	38.6 ± 2.1 e	28,744 ± 1040 b
LF	68.9 ± 5.2 a	1.2 ± 0.1 bcd	301 ± 4 a	70.1 ± 5.3 a	67.1 ± 0.2 b	2.4 ± 0.1 c	4319 ± 11 c	69.5 ± 0.4 b	23,777 ± 299 c

Table 3. *Cont.*

Treatments	NH_3 (% Total N Applied)	N_2O (% Total N Applied)	N (g m^{-2})	N (% Total N Applied)	CO_2 (% Total C Applied)	CH_4 (% Total C Applied)	C (g m^{-2})	C (% Total C Applied)	GWP (g CO_2-eq. m^{-2})
LF + Bioc	54.2 ± 2.7 b	1.0 ± 0.1 cde	206 ± 13 b	55.2 ± 2.8 b	53.9 ± 0.5 d	1.6 ± 0.2 d	4007 ± 60 cd	55.5 ± 0.8 d	20,726 ± 1003 de
LF + Alum	36.9 ± 4.9 cd	1.2 ± 0.1 bcd	152 ± 4 c	38.1 ± 5.0 cd	64.4 ± 3.1 bc	2.4 ± 0.1 c	3719 ± 40 def	66.8 ± 3.3 bc	20,703 ± 114 de
LF + Clin	45.5 ± 6.1 bc	1.4 ± 0.2 b	153 ± 1 c	46.8 ± 6.3 bc	65.3 ± 5.1 bc	2.4 ± 0.3 c	3973 ± 182 cde	67.8 ± 5.4 bc	21,883 ± 1089 cd
p slurries (A)	***	ns	***	***	***	***	***	***	***
p additives (B)	***	***	***	***	***	***	***	***	***
A × B	***	***	***	***	***	***	***	***	***

Note: $n = 3$: three replications per treatment. 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, respectively, not significant or significant at the 0.001 probability level. N: $NH_3 + N_2O$. C: $CO_2 + CH_4$. GWP: global warming potential expressed in CO_2 equivalents (CO_2 = 1, CH_4 = 28, direct N_2O = 265, indirect N_2O = 1% of NH_3-N volatilised).

Table 4. Average nitrous oxide fluxes (mg m^{-2} h^{-1}) and total flux (mg m^{-2}) from each treatment (mean ± standard deviation) ($n = 3$).

Treatments	Days of Experiment										Total Flux
	1	2	3–4	5–6	7–8	9–11	12–15	16–20	21–25	26–30	∑0–30
WS	8 ± 1 b	9 ± 1 b	8 ± 1 b	9 ± 1 b	8 ± 1 c	14 ± 1 a	9 ± 1 bcd	8 ± 1 cde	9 ± 1 cd	10 ± 1 c	7123 ± 4 cde
WS + Bioc	7 ± 1 ef	7 ± 1 d	7 ± 1 cd	7 ± 1 de	6 ± 1 d	12 ± 1 bc	7 ± 1 cd	6 ± 1 f	6 ± 1 d	7 ± 1 c	5389 ± 14 e
WS + Alum	8 ± 1 cd	8 ± 1 c	7 ± 1 c	7 ± 1 cd	7 ± 1 d	12 ± 1 bc	8 ± 1 bcd	7 ± 1 def	8 ± 1 cd	10 ± 1 c	6251 ± 75 cde
WS + Clin	7 ± 1 de	8 ± 1 c	7 ± 1 c	7 ± 1 cd	7 ± 1 d	11 ± 1 c	7 ± 1 bcd	7 ± 1 ef	7 ± cd	8 ± 1 c	5837 ± 64 de
SF	7 ± 1 cde	8 ± 1 c	8 ± 1 b	8 ± 1 b	9 ± 1 c	9 ± 1 d	14 ± 3 a	25 ± 1 a	71 ± 1 a	116 ± 1 a	28,546 ± 721 a
SF + Bioc	6 ± 1 g	6 ± 1 f	6 ± 1 d	6 ± 1 e	7 ± 1 d	7 ± 1 f	6 ± 1 d	7 ± 1 def	15 ± 3 bc	45 ± 4 b	10,209 ± 4 b
SF + Alum	6 ± 1 fg	7 ± 1 de	7 ± 1 cd	7 ± 1 de	7 ± 1 d	8 ± 1 ef	7 ± 1 bcd	7 ± 1 def	19 ± 1 b	8 ± 1 c	5563 ± 45 e
SF + Clin	6 ± 1 g	6 ± 1 ef	7 ± 1 d	7 ± 1 de	7 ± 1 d	7 ± 1 f	7 ± 1 cd	8 ± 1 def	10 ± 1 cd	44 ± 5 b	10,817 ± 1169 b
LF	10 ± 1 a	9 ± 1 a	9 ± 1 a	11 ± 1 a	12 ± 1 a	13 ± 1 b	11 ± 1 ab	10 ± 1 b	10 ± 1 cd	9 ± 1 c	7977 ± 51 c
LF + Bioc	7 ± 1 de	7 ± 1 d	7 ± 1 cd	8 ± 1 c	8 ± 1 c	9 ± 1 de	8 ± 1 bcd	7 ± 1 def	7 ± 1 cd	7 ± c	5660 ± 89 de
LF + Alum	10 ± 1 a	9 ± 1 a	9 ± 1 a	10 ± 1 a	11 ± 1 ab	12 ± 1 bc	10 ± abc	10 ± 1 bc	10 ± 1 cd	9 ± 1 c	7574 ± 431 cd
LF + Clin	8 ± 1 bc	9 ± 1 a	9 ± 1 a	10 ± 1 a	11 ± 1 b	11 ± 1 c	9 ± 1 bcd	9 ± 1 bcd	9 ± 1 cd	8 ± 1 c	6958 ± 91 cde
p slurries (A)	***	***	***	***	***	***	**	***	***	***	***
p additives (B)	***	***	***	***	***	***	***	***	***	***	***
A × B	***	***	***	***	***	***	**	***	***	***	***

Note: $n = 3$: three replications per treatment. Values from the interaction slurries × additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. ** and *** mean that the factor or interaction effects were, respectively, significant at the 0.01 and 0.001 probability level.

The nitrification and denitrification processes are the source of N_2O emissions by the presence of aerobic and anaerobic conditions in the stored slurries, but only when a dry crust has formed on the surface [28]. In this study (Table 4), the N_2O fluxes in the WS and LF treatments with and without additives did not vary greatly during the 30 d of the experiment while the N_2O fluxes in SF treatments increased up to day 16. Such an increase in N_2O fluxes from SF treatments may be related to water evaporation and dry conditions together with air filled porosity, which enhanced a mosaic of anaerobic and aerobic micro-sites [29]. As can be seen in Table 3, the N_2O emissions were reduced by 70% by the addition of the additives (Bioc, Alum and Clin) in SF treatments, but without significant reductions in WS or LS treatments. The addition of additives (Bioc, Alum and Clin) before mechanical separation of the WS produced an SF very rich in additives, comparative to the LF and WS and, thus, led to significant N_2O reductions that varied from 60 to 80% for all additives. The decrease in N_2O emissions may be related with the adsorption of NH_4^+ by biochar or clinoptilolite that reduced their availability for nitrification [22], and the low pH by alum that inhibited the nitrification/denitrification processes [17,30]. For the three additives (Bioc, Alum and Clin), results of this study (70% N_2O reduction for 5% w/w of each additive) are in line with previous studies, where Brennan et al. [31] reported that cattle slurry amended with biochar (12% w/w) reduced N_2O loss by 63%, Wang et al. [32] found that pig manure amended with biochar (10% w/w) mixed with clinoptilolite reduced N_2O loss by approximately 80%, and Regueiro et al. [17] reported that SF of pig slurry amended with alum (2% w/w) reduced N_2O loss by 79%.

The N (NH_3 + N_2O) emissions, expressed as g m^{-2} or as % of applied N, increased significantly ($p < 0.05$) in the following order: SF > WS > LF, with a reduction of approximately 60% for the SF treatment and an increase of approximately 36% for the LF treatment, when compared with the WS treatment (Table 3). The cumulative NH_3 emissions, expressed as % of total N applied, were significantly reduced ($p < 0.05$) in all additive treatments (Bioc, Alum and Clin) relative to the same treatments without additives (WS, SF and LF), with reductions of 52% for the additive Alum and 36% for the additives Bioc and Clin (Table 3).

The NH_3 emissions did not differ significantly ($p > 0.05$) among the separated fractions together (SF and LF) and the WS, which is not in agreement with previous studies [13,29] that state that NH_3 emissions could increase when raw slurry was separated. Comparative to the application of WS (100% emission), slurry separation alone (LF vs. SF) did not significantly increase ($p > 0.05$) NH_3, N_2O and N emissions (Table 5). The combination of the slurry separation with the additives (Bioc, Alum and Clin) did not significantly reduce ($p > 0.05$) NH_3, N_2O and N emissions (Table 5).

Table 5. Effect of different additives and slurry separation on the balance of gaseous losses compared with the whole slurry (as % of emissions observed in the whole slurry) (mean ± standard deviation) ($n = 3$).

Parameters	Whole Slurry (%)	Slurry Separation (%)	Slurry Separation + Biochar (%)	Slurry Separation + Alum (%)	Slurry Separation + Clinoptilolite (%)
NH_3	100 ± 1 a	100 ± 15 a	117 ± 9 a	113 ± 11 a	101 ± 14 a
N_2O	100 ± 1 a	123 ± 14 a	95 ± 8 a	95 ± 12 a	100 ± 17 a
N	100 ± 1 a	101 ± 15 a	116 ± 9 a	112 ± 11 a	101 ± 14 a
CO_2	100 ± 1 a	72 ± 4 bc	78 ± 1 b	59 ± 5 d	69 ± 1 c
CH_4	100 ± 1 a	68 ± 5 bc	81 ± 4 b	68 ± 12 bc	58 ± 5 c
C	100 ± 1 a	72 ± 4 bc	78 ± 1 b	60 ± 5 d	68 ± 2 c
GWP	100 ± 1 c	117 ± 2 a	109 ± 1 b	105 ± 3 bc	103 ± 1 bc

Note: $n = 3$: three replications per treatment. Values presented with different lowercase letters within rows and are significantly different ($p < 0.05$) by Tukey test. N: NH_3 + N_2O. C: CO_2 + CH_4. GWP: global warming potential expressed in CO_2 equivalents (CO_2 = 1, CH_4 = 28, direct N_2O = 265, indirect N_2O = 1% of NH_3-N volatilised).

3.3. Carbon Emissions

Comparative to other treatment slurries, the daily CO_2 fluxes were significantly higher ($p < 0.05$) in the first 2 d of experiment for the LF treatment and between day 9 and the end of the experiment for the WS treatment (Table 6). The daily CO_2 fluxes were reduced, but not always significantly, in treatments with the additives (Bioc, Alum and Clin) when compared with the same treatments without additives (Table 6). On most measurement dates, the daily CO_2 fluxes from treatments with additives were reduced significantly ($p < 0.05$) in the following order: Alum < Bioc < Clin, with a reduction of approximately 20% for treatments with Bio and Alum (Table 6). The cumulative CO_2 emissions, expressed in g m^{-2}, were not significantly different ($p > 0.05$) among SF and LF treatments, being significantly lower ($p < 0.05$) by approximately 25% than the WS treatment (Table 6). The cumulative CO_2 emissions, expressed in g m^{-2}, were reduced in all additive treatments (Bioc, Alum and Clin) relative to the same treatments without additives (WS, SF and LF), with a significant decrease of 22% for the additives Bioc and Alum (Table 6). The cumulative CO_2 emissions, expressed as % of total C applied, were reduced significantly ($p < 0.05$) in the following order: SF < LF < WS, with a reduction of 61% in the SF treatment relative to WS (Table 3). The cumulative CO_2 emissions, expressed as % of total C applied, were significantly reduced ($p < 0.05$) by 25% in all treatments with the additive Bioc and by 33% in the SF treatment with Alum (Table 3).

The two main sources of CO_2 emissions are the microbial degradation of organic matter and urea hydrolysis [33]. In addition, it will be expected that the CO_2 emissions are higher for SF since these losses seem higher in slurry fractions with high amounts of C [13], but this patten is not always reported in other studies [12,34]. The high CO_2 emissions obtained in WS and LF relative to SF could be related with the release of the CO_2 dissolved in the slurry itself and/or bicarbonate and carbonate present in the slurries [35]. Moreover, the SF had dissolved CO_2 and very low amounts of water-soluble C together with the reduction in volume by water loss and aerobic condition by sample compaction [17]. As can be seen in Table 3, the CO_2 emissions were reduced significantly by 25% by the addition of biochar. The results of this study are lower than previous studies [21,32], which reported that CO_2 emissions from animal slurry were reduced by between 34 and 50% by the addition of biochar (5–10% *w*/*w*), due to either sorption onto the biochar or a reduction in the labile C availability. However, the additive clinoptilolite appears to have had no effect on CO_2 emission in this study (Table 3), which is in line with a previous study [21] that reported the absence of significant effect of this additive (2.5% *w*/*w*) on CO_2 reduction. In this study, the decrease in CO_2 emission by alum added to SF (33% CO_2 reduction for 5% *w*/*w* of alum) was because most of the dissolved CO_2 is lost during the acidification process [30], which is in line with Regueiro et al. [17], who reported that the SF of pig slurry amended with alum (2% *w*/*w*) reduced CO_2 loss by 41%.

During most measurement days, the daily CH_4 fluxes decreased progressively in treatments as the experiment progressed and are shown in Table 7. The daily CH_4 fluxes were significantly reduced ($p < 0.05$) by approximately 46% in SF and LF treatments, when compared with the WS treatment (Table 7). The daily CH_4 fluxes were reduced, but not always significantly, in treatments with the additives (Bioc, Alum and Clin), when compared with the same treatments without additives (Table 7). On most measurement dates, the daily CH_4 fluxes from the treatments with additives were reduced significantly ($p < 0.05$) in the following order: Bioc < Alum < Clin, with a reduction of approximately 30% for treatments with Bio and Alum (Table 7). The cumulative CH_4 emissions, expressed in g m^{-2}, were not significantly different ($p > 0.05$) among SF and LF treatments, being significantly lower ($p < 0.05$) by approximately 45% than the WS treatment (Table 7). Comparative to the treatments without the additives (WS, SF and LF), the cumulative CH_4 emissions, expressed in g m^{-2}, from the additives Bioc and Alum, resulted in a reduction of 41%, although not statistically significant (Table 3). The cumulative CH_4 emissions, expressed as % of total C applied, were significantly higher ($p < 0.05$) in the following order: WS > LF > SF, with a reduction of 49% in SF relative to WS (Table 3). The cumulative CH_4 emissions, expressed as % of total C applied, were significantly reduced ($p < 0.05$) by 50% by the additive Bioc and by 30% by the additive Alum, although not always statistically significant for Alum (Table 3).

Table 6. Average carbon dioxide fluxes (g m^{-2} h^{-1}) and total flux (g m^{-2}) from each treatment (mean ± standard deviation) (n = 3).

Treatments	**Days of Experiment**										**Total Flux**
	1	**2**	**3–4**	**5–6**	**7–8**	**9–11**	**12–15**	**16–20**	**21–25**	**26–30**	**∑0–30**
WS	21.0 ± 0.7 bc	22.4 ± 1.5 b	21.8 ± 0.4 abc	22.1 ± 0.2 ab	21.8 ± 0.1 a	24.2 ± 0.3 a	26.4 ± 1.0 a	27.7 ± 1.6 a	32.5 ± 0.2 a	30.0 ± 1.4 a	20,168 ± 61 a
WS + Bioc	16.5 ± 0.3 ef	16.4 ± 0.2 f	16.6 ± 0.3 d	16.9 ± 0.4 fgh	16.7 ± 0.5 de	17.6 ± 0.8 f	18.4 ± 1.0 def	21.0 ± 2.5 bc	22.3 ± 1.9 b	26.3 ± 1.4 a	15,181 ± 634 c
WS + Alum	15.8 ± 0.1 f	15.6 ± 0.1 f	15.6 ± 0.1 d	15.9 ± 0.1 hi	15.3 ± 0.1 e	15.8 ± 0.1 g	16.4 ± 0.1 f	16.4 ± 0.1 c	18.2 ± 0.2 c	19.3 ± 0.3 b	12,753 ± 69 e
WS + Clin	18.8 ± 0.4 cd	19.4 ± 0.9 cde	19.1 ± 0.5 bcd	19.2 ± 0.6 cd	18.5 ± 0.6 bc	19.6 ± 0.6 cde	21.7 ± 0.4 bc	24.8 ± 0.3 ab	30.2 ± 0.4 a	29.5 ± 1.2 a	18,029 ± 435 b
SF	21.7 ± 0.1 b	20.6 ± 0.1 bcd	18.7 ± 0.1 bcd	18.5 ± 0.2 de	19.3 ± 0.2 b	22.0 ± 0.1 b	21.4 ± 0.1 bc	19.3 ± 0.1 c	18.3 ± 0.1 c	19.1 ± 0.1 b	14,949 ± 18 c
SF + Bioc	17.1 ± 1.0 def	17.3 ± 0.7 ef	16.1 ± 0.4 d	16.1 ± 0.4 ghi	17.1 ± 0.3 cd	21.6 ± 0.1 b	19.5 ± 0.1 cde	17.8 ± 0.3 c	16.6 ± 0.2 c	17.6 ± 0.4 b	13,528 ± 143 de
SF + Alum	16.1 ± 0.1 ef	15.9 ± 0.1 f	15.1 ± 0.1 d	15.1 ± 0.1 i	15.8 ± 0.1 de	18.4 ± 0.1 ef	18.0 ± 0.1 def	17.1 ± 0.1 c	16.5 ± 0.1 c	17.3 ± 0.1 b	12,760 ± 1 e
SF + Clin	18.9 ± 0.4 cd	18.1 ± 0.2 def	15.8 ± 0.4 d	15.7 ± 0.4 hi	16.9 ± 0.4 d	20.4 ± 0.3 bcd	22.0 ± 0.4 b	19.8 ± 0.3 c	17.8 ± 0.4c	19.0 ± 0.3 b	14,297 ± 248 cd
LF	26.5 ± 0.3 a	25.7 ± 0.4 a	24.8 ± 0.2 a	23.1 ± 0.1 a	21.1 ± 0.1 a	20.8 ± 0.1 bc	20.0 ± 0.1 bcde	18.0 ±0.1 c	17.7 ± 0.1 c	17.6 ± 0.1 b	15,278 ± 12 c
LF + Bioc	21.6 ± 0.6 b	21.8 ± 0.6 bc	21.9 ± 0.5 ab	20.6 ± 0.4 bc	19.1 ± 0.2 b	18.9 ± 0.1 def	17.8 ± 0.2 def	17.1 ± 0.2 c	17.9 ± 0.2 c	18.6 ± 0.1 b	14,260 ± 90 cd
LF + Alum	18.2 ± 0.1 de	17.7 ± 0.1 def	17.2 ± 0.1 cd	17.5 ± 0.1 efg	16.9 ± 0.1 d	17.5 ± 0.1 fg	17.5 ± 0.1 ef	17.2 ± 0.1 c	17.3 ± 0.1 c	17.1 ± 0.1 b	13,152 ± 4 de
LF + Clin	20.5 ± 0.4 bc	18.2 ± 0.7 def	14.6 ± 2.9 d	18.3 ± 0.3 def	18.5 ± 0.2 bc	20.5 ± 0.3 bcd	20.2 ± 0.6 bcd	18.8 ± 0.5 c	17.6 ± 0.5 c	17.8 ± 0.5 b	14,047 ± 362 cde
p slurries (A)	***	***	***	***	***	***	***	***	***	***	***
p additives (B)	***	***	***	***	***	***	***	***	***	***	***
A × B	***	***	***	***	***	***	***	***	***	***	***

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. *** means that the factor or interaction effects were significant at the 0.001 probability level.

Table 7. Average methane fluxes (mg m^{-2} h^{-1}) and total flux (mg m^{-2}) from each treatment (mean ± standard deviation) (n = 3).

Treatments	**Days of Experiment**										**Total Flux**
	1	**2**	**3–4**	**5–6**	**7–8**	**9–11**	**12–15**	**16–20**	**21–25**	**26–30**	**∑0–30**
WS	246 ± 16 b	494 ± 85 a	420 ± 14 a	520 ± 23 a	520 ± 18 a	722 ± 47 ab	1105 ± 48 a	771 ± 121 a	373 ± 25 ab	240 ± 24 cd	434,233 ± 34,931 a
WS + Bioc	56 ± 4 f	75 ± 8 de	122 ± 26 efg	174 ± 59 cde	283 ± 123 bcde	554 ± 197 bc	179 ± 23 g	142 ± 11 d	130 ± 33 e	122 ± 31 ef	142,747 ± 18,963 gh
WS + Alum	99 ± 7 de	70 ± 5 de	71 ± 5 g	55 ± 7 f	87 ± 12 f	195 ± 13 e	197 ± 13 g	241 ± 12 cd	185 ± 11 de	179 ± 11 def	124,662 ± 7934 h
WS + Clin	104 ± 2 de	220 ± 40 bc	254 ± 17 cd	213 ± 18 bcd	439 ± 12 ab	963 ± 33 a	773 ± 33 b	353 ± 23 bc	122 ± 4 e	116 ± 4 ef	280,299 ± 9174 b
SF	135 ± 1 d	145 ± 3 cde	162 ± 1 ef	177 ± 1 cde	229 ± 1 cdef	284 ± 1 cde	427 ± 5 c	456 ± 3 b	372 ± 2 ab	403 ± 11 b	251,122 ± 1968 bcd
SF + Bioc	71 ± 2 ef	63 ± 4 e	56 ± 8 g	90 ± 23 ef	223 ± 36 cdef	255 ± 16 e	214 ± 19 fg	210 ± 19 cd	295 ± 57 bcd	258 ± 27 cd	156,615 ± 18,826 fgh
SF + Alum	115 ± 16 d	118 ± 15 cde	66 ± 8 g	63 ± 8 f	138 ± 13 ef	193 ± 18 e	207 ± 16 fg	215 ± 19 cd	326 ± 31 bc	330 ± 31 bc	161,732 ± 15,311 fgh
SF + Clin	63 ± 2 ef	75 ± 2 de	100 ± 1 fg	149 ± 6 def	378 ± 14 abc	538 ± 29 bcd	296 ± 7 def	311 ± 3 bcd	461 ± 9 a	513 ± 10 a	261,722 ± 1768 bc
LF	363 ± 11 a	331 ± 9 b	332 ± 8 b	263 ± 8 bc	268 ± 5 bcde	337 ± 12 cde	363 ± 3 cd	347 ± 6 bc	278 ± 10 bcd	188 ± 7 def	227,903 ± 5691 bcde
LF + Bioc	188 ± 5 c	224 ± 18 bc	232 ± 23 d	262 ± 37 bc	191 ± 24 def	272 ± 31 de	393 ± 55 cd	362 ± 24 bc	104 ± 10 e	86 ± 1 f	177,253 ± 15,121 efgh
LF + Alum	247 ± 13 b	198 ± 14 bcd	186 ± 12 de	150 ± 17 def	215 ± 9 cdef	234 ± 12 e	253 ± 1 efg	296 ± 11 bcd	312 ± 16 bcd	317 ± 17 bc	197,900 ± 1702 defg
LF + Clin	195 ± 1 c	188 ± 6 cde	310 ± 10 bc	304 ± 4 b	339 ± 5 bcd	308 ± 2 cde	321 ± 8 de	382 ± 19 bc	210 ± 25 cde	204 ± 28 de	213,937 ± 10,473 cdef
p slurries (A)	***	***	***	***	**	***	***	*	***	***	***
p additives (B)	***	***	***	***	***	***	***	***	***	***	**
A × B	***	***	***	***	***	***	***	***	***	***	***

Note: n = 3: three replications per treatment. Values from the interaction slurries × additives are presented with different lowercase letters within columns and are significantly different ($p < 0.05$) by Tukey test. *, ** and *** mean that the factor or interaction effects were, respectively, significant at the 0.05, 0.01 and 0.001 probability level.

The emission of CH_4 from slurries is related to the degradation of organic matter in anaerobic conditions [36]. In this study, the CH_4 emissions were significantly higher in WS and LF relative to SF, in line with previous studies [13,17], being related, as previously explained for CO_2 losses, with the higher level of readily degradable C present in WS and LF. The results of this study are comparable to previous studies [21,32,37], which found that CH_4 emissions from pig slurry were reduced by between 50 and 61% by the addition of biochar (2.5–10.0% w/w), which can be explained by their adsorption ability. Furthermore, the addition of clinoptilolite did not affect CH_4 losses (Table 3), which is in agreement with Pereira et al. [21], who found no effect of this additive (2.5% w/w) on CH_4 emissions. The addition of alum could change the methanogenic activity because this process is usually inhibited at $pH < 6.0$ [38]. In this study, since the CO_2 emissions occurred mainly under aerobic conditions, significant CH_4 emissions were not expected, with the exception of the SF with alum, where CH_4 losses were significantly reduced ($p < 0.05$) by 70% (Table 3). However, the results of this study (30% CH_4 reduction for 5% w/w of alum) are lower than Regueiro et al. [17], who reported that CH_4 emissions from pig slurry were reduced by between 81 and 92% by the addition of alum (2% w/w).

The cumulative C ($CO_2 + CH_4$) emissions, expressed in g m^{-2}, were not significantly different ($p > 0.05$) among SF and LF treatments, being significantly lower ($p < 0.05$) by approximately 45% than the WS treatment (Table 3). The cumulative CH_4 emissions, expressed in g m^{-2}, were not significantly different ($p > 0.05$) among WS and LF treatments, being significantly lower ($p < 0.05$) by approximately 74% than the SF treatment (Table 3). The cumulative C ($CO_2 + CH_4$) emissions, expressed as % of total C applied, were reduced significantly ($p < 0.05$) in the following order: SF < LF < WS, with a reduction of 60% in the SF treatment relative to WS (Table 3). The cumulative C ($CO_2 + CH_4$) emissions, expressed as % of total C applied, were significantly reduced ($p < 0.05$) by 26% in treatments with the additive Bioc, when compared with all other treatments with or without additives (Table 3). The cumulative GWP emissions, expressed as CO_2 eq. m^{-2}, were significantly increased ($p < 0.05$) by approximately 67% in the SF treatment, when compared with WS and LF treatments (Table 3). The cumulative GWP emissions, expressed as CO_2 eq. m^{-2}, were significantly reduced ($p < 0.05$) by approximately 28%, respectively, in treatments with the additives Bio and Alum when compared with all other treatments with or without additives (Table 3).

Comparative to the application of WS (100% emission), slurry separation alone (LF vs. SF) significantly reduced ($p < 0.05$) CO_2 or C emissions by 29%, and CH_4 by 40% (Table 5). The separation alone significantly increased ($p < 0.05$) the GWP emissions of separated fractions together (SF and LF). The combination of the slurry separation with the additives (Bioc, Alum and Clin) did not significantly reduce ($p > 0.05$) CH_4 emissions, whereas the CO_2 and C emissions were significantly reduced ($p < 0.05$) by approximately 40% with the additive Alum (Table 5). However, when additives (Bioc, Alum and Clin) were applied before separation, the GWP emissions of the fractions combined together (SF and LF) were significantly lower ($p < 0.05$) than from WS.

4. Conclusions

The results indicated that the mechanical separation of the WS modified the initial physicochemical characteristics and increased the GWP emissions of the two separated fractions together (solid and liquid fractions), but not the NH_3 losses. However, the addition of the additives (biochar, alum or clinoptilolite) before mechanical separation increased the fertilizer value and reduced the GWP emissions from the solid and liquid fractions. Additionally, just the additive alum was effective in the reduction of *E. coli*. The addition of the three additives led to significant reductions in NH_3 and N_2O emissions, with higher reductions in NH_3 losses for alum observed and similar N_2O losses for all additives, whereas the CO_2 and CH_4 emissions were reduced by biochar and alum, but not by clinoptilolite.

Globally, the addition of alum before mechanical separation has the potential to be the most effective mitigation measure because it improved the fertilizer value and sanitation and decreased the gaseous losses from pig slurry management when compared with biochar and clinoptilolite. Thus, farm scale studies are needed to validate these results under real conditions.

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References

1. Montes, F.; Meinen, R.; Dell, C.; Rotz, A.; Hristov, A.N.; Oh, J.; Waghorn, G.; Gerber, P.J.; Henderson, B.; Makkar, H.P.S. Mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management options. *J. Anim. Sci.* **2013**, *91*, 5070–5094. [CrossRef] [PubMed]
2. Chadwick, D.; Sommer, S.; Thorman, R.; Fangueiro, D.; Cardenas, L.; Amon, B.; Misselbrook, T. Manure management: Implications for greenhouse gas emissions. *Anim. Feed Sci. Technol.* **2011**, *166*, 514–531. [CrossRef]
3. Sommer, S.G.; Zhang, G.Q.; Bannink, A.; Chadwick, D.; Misselbrook, T.; Harrison, R.; Hutchings, N.J.; Menzi, H.; Monteny, G.J.; Ni, J.Q.; et al. Algorithms determining ammonia emission from buildings housing cattle and pigs and from manure stores. *Adv. Agron.* **2006**, *89*, 261–335. [CrossRef]
4. Hou, Y.; Velthof, G.L.; Oenema, O. Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: A meta-analysis and integrated assessment. *Glob. Chang. Biol.* **2015**, *21*, 1293–1312. [CrossRef]
5. Hou, Y.; Velthof, G.L.; Lesschen, J.P.; Staritsky, I.G.; Oenema, O. Nutrient recovery and emissions of ammonia, nitrous oxide and methane from animal manure in Europe: Effects of manure treatment technologies. *Environ. Sci. Technol.* **2017**, *51*, 375–383. [CrossRef]
6. Sajeev, E.P.M.; Winiwarter, W.; Amon, B. Greenhouse gas and ammonia emissions from different stages of liquid manure management chains: Abatement options and emission interactions. *J. Environ. Qual.* **2018**, *47*, 30–41. [CrossRef]
7. Foged, H.L.; Flotats, X.; Blasi, A.B.; Palatsi, J.; Magri, A.; Schelde, K.M. *Inventory of Manure Processing Activities in Europe. Technical Report No. I Concerning "Manure Processing Activities in Europe" to the European Commission. Directorate-General Environment, Project Reference: ENV.B.1/ETU/2010/0007*; Agro Business Park: Brussels, Belgium, 2011; 138p.
8. Bittman, S.; Dedina, M.; Howard, C.M.; Oenema, O.; Sutton, M.A. (Eds.) *Options for Ammonia Mitigation: Guidance from the UNECE Task Force on Reactive Nitrogen*; *Project Reference: CEH Project no. C04910*; NERC/Centre for Ecology & Hydrology: Edinburgh, UK, 2014; 83p.
9. UNECE. UNECE Guidance Document on Sustainable Nitrogen Management. United Nations Economic Commission for Europe (UNECE) Convention on Long-Range Transboundary Air Pollution (Air Convention). ECE/EB.AIR/149. 2020. 198p. Available online: https://unece.org/sites/default/files/2021-08/ECE_EB.AIR_149-2104922E_0.pdf (accessed on 10 May 2022).
10. McCrory, D.F.; Hobbs, P.J. Additives to reduce ammonia and odor emissions from livestock wastes: A review. *J. Environ. Qual.* **2001**, *30*, 345–355. [CrossRef]
11. Van der Stelt, B.; Temminghoff, E.J.M.; Van Vliet, P.C.J.; Van Riemsdijk, W.H. Volatilization of ammonia from manure as affected by manure additives, temperature and mixing. *Bioresour. Technol. Rep.* **2007**, *98*, 3449–3455. [CrossRef]
12. Fangueiro, D.; Senbayran, M.; Trindade, H.; Chadwick, D. Cattle slurry treatment by screw press separation and chemically enhanced settling: Effect on greenhouse gas emissions after land spreading and grass yield. *Bioresour. Technol.* **2008**, *99*, 7132–7142. [CrossRef]
13. Fangueiro, D.; Coutinho, J.; Chadwick, D.; Moreira, N.; Trindade, H. Effect of cattle slurry separation on greenhouse gas and ammonia emissions during storage. *J. Environ. Qual.* **2008**, *37*, 2322–2331. [CrossRef]

14. Hjorth, M.; Christensen, K.V.; Christensen, M.L.; Sommer, S.G. Solid-liquid separation of animal slurry in theory and practice. A review. *Agron. Sustain. Dev.* **2010**, *30*, 153–180. [CrossRef]
15. Guilayn, F.; Jimenez, J.; Rouez, M.; Crest, M.; Patureau, D. Digestate mechanical separation: Efficiency profiles based on anaerobic digestion feedstock and equipment choice. *Bioresour. Technol.* **2018**, *274*, 180–189. [CrossRef]
16. Popovic, O.; Hjorth, M.; Jensen, L. Phosphorus, copper and zinc in solid and liquid fractions from full-scale and laboratory-separated pig slurry. *Environ. Technol.* **2012**, *33*, 2119–2131. [CrossRef] [PubMed]
17. 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. [CrossRef]
18. Pereira, J.; Fangueiro, D.; Misselbrook, T.H.; Chadwick, D.R.; Coutinho, J.; Trindade, H. Ammonia and greenhouse gas emissions from slatted and solid floors in dairy cattle houses: A scale model study. *Biosyst. Eng.* **2011**, *109*, 148–157. [CrossRef]
19. Pereira, J.; Misselbrook, T.H.; Chadwick, D.R.; Coutinho, J.; Trindade, H. Effects of temperature and dairy cattle excreta characteristics on potential ammonia and greenhouse gas emissions from housing: A laboratory study. *Biosyst. Eng.* **2012**, *112*, 138–150. [CrossRef]
20. Soares, A.S.; Miranda, C.; Teixeira, C.A.; Coutinho, J.; Trindade, H.; Coelho, A.C. Impact of different treatments on Escherichia coli during storage of cattle slurry. *J. Environ. Manag.* **2019**, *236*, 323–327. [CrossRef]
21. Pereira, J.L.S.; Figueiredo, V.; Pinto, A.; Pinto, A.F.M.A.; Silva, M.E.F.; Brás, I.; Perdigão, A.; Wessel, D.F. Effects of biochar and clinoptilolite on composition and gaseous emissions during the storage of separated liquid fraction of pig slurry. *Appl. Sci.* **2020**, *10*, 5652. [CrossRef]
22. Pereira, J.L.S.; Perdigão, A.; Marques, F.; Coelho, C.; Mota, M.; Fangueiro, D. Evaluation of Tomato-Based Packing Material for Retention of Ammonia, Nitrous Oxide, Carbon Dioxide and Methane in Gas Phase Biofilters: A Laboratory Study. *Agronomy* **2021**, *11*, 360. [CrossRef]
23. IPCC. *2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories*; Calvo Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize, S., Osako, A., Pyrozhenko, Y., Shermanau, P., Federici, S., Eds.; IPCC: Geneva, Switzerland, 2019; Available online: http://www.ipcc-nggip.iges.or.jp (accessed on 10 May 2022).
24. Lefcourt, A.M.; Meisinger, J.J. Effect of adding alum or zeolite to dairy slurry on ammonia volatilization and chemical composition. *J. Dairy Sci.* **2001**, *84*, 1814–1821. [CrossRef]
25. Rodrigues, J.; Alvarenga, P.; Silva, A.C.; Brito, L.; Tavares, J.; Fangueiro, D. Animal Slurry Sanitization through pH Adjustment: Process Optimization and Impact on Slurry Characteristics. *Agronomy* **2021**, *11*, 517. [CrossRef]
26. Clough, T.J.; Condron, L.M.; Kammann, C.; Müller, C. A review of biochar and soil nitrogen dynamics. *Agronomy* **2013**, *3*, 275–293. [CrossRef]
27. Kalus, K.; Koziel, J.A.; Opaliński, S. A review of biochar properties and their utilization in crop agriculture and livestock production. *Appl. Sci.* **2019**, *9*, 3494. [CrossRef]
28. Berg, W.; Brunsch, R.; Pazsiczki, I. Greenhouse gas emissions from covered slurry compared with uncovered during storage. *Agric. Ecosyst. Environ.* **2006**, *112*, 129–134. [CrossRef]
29. Dinuccio, E.; Berg, W.; Balsari, P. Gaseous emissions from the storage of untreated slurries and the fractions obtained after mechanical separation. *Atmos. Environ.* **2008**, *42*, 2448–2459. [CrossRef]
30. Fangueiro, D.; Surgy, S.; Coutinho, J.; Vasconcelos, E. Impact of cattle slurry acidification on carbon and nitrogen dynamics during storage and after soil incorporation. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 540–550. [CrossRef]
31. Brennan, R.B.; Healy, M.G.; Fenton, O.; Lanigan, G.J. The effect of chemical amendments used for phosphorus abatement on greenhouse gas and ammonia emissions from dairy cattle slurry: Synergies and pollution swapping. *PLoS ONE* **2015**, *10*, e0111965. [CrossRef]
32. Wang, Q.; Awasthi, M.K.; Ren, X.N.; Zhao, J.C.; Li, R.H.; Wang, Z.; Wang, M.J.; Chen, H.Y.; Zhang, Z.Q. Combining biochar, zeolite and wood vinegar for composting of pig manure: The effect on greenhouse gas emission and nitrogen conservation. *Waste Manag.* **2018**, *74*, 221–230. [CrossRef]
33. Moset, V.; Cambra-Lopez, M.; Estelles, F.; Torres, A.G.; Cerisuelo, A. Evolution of chemical composition and gas emission from aged pig slurry during outdoor storage with and without prior solid separation. *Biosyst. Eng.* **2012**, *3*, 2–10. [CrossRef]
34. Fangueiro, D.; Chadwick, D.; Dixon, L.; Bol, R. Quantification of priming and CO_2 respiration sources following the application of different slurry particle size fractions to a grassland soil. *Soil Biol. Biochem.* **2007**, *39*, 2608–2620. [CrossRef]
35. Flessa, H.; Beese, F. Laboratory estimates of trace gas emissions following surface application and injection of cattle slurry. *J. Environ. Qual.* **2000**, *29*, 262–268. [CrossRef]
36. Sommer, S.G.; Petersen, S.O.; Sorensen, P.; Poulsen, H.D.; Moller, H.B. Methane and carbon dioxide emissions and nitrogen turnover during liquid manure storage. *Nutr. Cycl. Agroecosyst.* **2007**, *78*, 27–36. [CrossRef]
37. Mao, H.; Zhang, H.Y.; Fu, Q.; Zhong, M.Z.; Li, R.H.; Zhai, B.N.; Wang, Z.H.; Zhou, L.N. Effects of four additives in pig manure composting on greenhouse gas emission reduction and bacterial community change. *Bioresour. Technol.* **2019**, *292*, 121896. [CrossRef] [PubMed]
38. Petersen, S.O.; Højberg, O.; Poulsen, M.; Schwab, C.; Eriksen, J. Methanogenic community changes, and emissions of methane and other gases, during storage of acidified and untreated pig slurry. *J. Appl. Microbiol.* **2014**, *117*, 160–172. [CrossRef] [PubMed]