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Aerated Buffalo Slurry Improves Spinach Plant Growth and Mitigates CO_2 and N_2O Emissions from Soil

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Abstract: Manure management is the main strategy for mitigating gas emissions from livestock farming. In this study, a laboratory-scale experiment was set up to identify suitable conditions to be applied in a farm-scale experiment. The liquid fraction (LF) of slurry was aerobically treated and greenhouse gas emissions from soil were evaluated. Furthermore, the value of treated LF as a fertilizer on spinach plants was also tested. The aeration of LF determined an increase in mean alkalinity due to ammonia loss. The mass fraction of heavy metals also decreased, likely due to the reduction in solubility. After being applied on soil, aerated LF determined lower CO_2 and N_2O emissions compared to untreated LF due to a reduced nitrogen load. Spinach plants fertilized with treated LF showed a lush growth and exhibited a lower heavy metal mass fraction as well as a higher content of antioxidants compared to plants fertilized with untreated slurry. Our results show that aeration might be an effective alternative for slurry management as it is able to produce an eco-friendly final product with a high fertilizing value.

Keywords: slurry aeration; seed germination; plant growth; soil GHGs; animal waste management

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1. Introduction

Livestock farming produces large volumes of manure consisting of a mixture of animal feces, urine, bedding materials, and other materials associated with animal waste production. Manure storage in tanks and slurry spreading in the field involve the production and the emission into the atmosphere of ammonia (NH_3) and greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), which have a negative impact on the climate and environment.

In the last decade, many studies have been carried out in order to find innovative solutions able to mitigate the impact of livestock farming on GHG emissions, including covering slurry tanks, manure composting, acidification, anaerobic digestion, solid–liquid separation, dilution, and slurry aeration [1,2]. Other practices concern waste disposal on the field such as the addition of chemical additives [3] and the injection of slurry into the soil [4]. Aeration is an alternative practice to treat slurry. It consists of the biological oxidation of degradable organic matter in which the continuous supply of oxygen by aerobic bacteria results in the production of CO_2 and water as by-products. Laboratory- and pilot-scale studies showed that slurry aeration may greatly increase NH_3 and N_2O emissions [5–7],

also during its successive disposal on the field [7], even if the emissions for both NH_3 and N_2O might be mitigated through low-flow phased oxic/anoxic treatment [8]. If the aeration process is properly controlled, the supply of oxygen can represent an alternative economic method compared to other more expensive ones, such as anaerobic digestion, in mitigating GHG emissions during manure storage and after its disposal on the field. The aired slurry is characterized by a lower ammonia load but greater nitrate content than untreated or anaerobic-treated slurry [9]; thus, its disposal in the field may determine a reduction in NH_3 and N_2O emissions if it is applied to soil in adequate volumes, which does not favor denitrification.

One of the problems associated with the disposal of slurry in the field is the risk of soil contamination by heavy metals [10] and other xenobiotic compounds, which may be a potential hazard for crops. Phenolic acids in the urine of animals, for instance, inhibit seed germination and early plant growth of different plant species [11]. Some heavy metals present in slurry such as Pb, Cu, Cd, Zn, and Cr can be absorbed by crops and thus enter into the food chain with important consequences on the top organisms. The aerobic treatment of slurry might reduce the presence of dangerous compounds and might promote an increase in alkalinity through ammonia removal, thus likely favoring heavy metals' precipitation [12]. To date, no literature is available on the effect that aeration treatment may have on heavy metals in slurry.

Although the aeration of slurry might favor an increase in GHG emissions during slurry storage, this phenomenon might be overcompensated by reduced emissions after the field application [2]. Therefore, the discussion on effects of treatments on emissions from slurry storage must take into account the context of good management practices along the whole manure management chain [2]. However, at present, the research focusing on this special issue is still limited [7], and no study is provided on the potential use of the aerated liquid fraction (LF) of slurry as a fertilizer. This study aimed to investigate the effectiveness of the aerobic treatment of buffalo slurry in reducing soil CO_2 and N_2O emissions following its application on soil and the promising use of the aerated liquid fraction of slurry in improving the agronomic traits of spinach plants. In this paper, we hypothesize that aeration, by altering the chemical–physical characteristics of the liquid fraction of buffalo slurry, may positively impact on soil GHG emissions, spinach growth, and the mass fraction of heavy metals in plants.

2. Materials and Methods

2.1. Chemical–Physical Characteristics of Liquid Fraction of Slurry

Liquid fraction of slurry, deriving from buffalo manure mechanically separated in solid and liquid fractions, was collected in flasks from open storage tanks and cold stored until laboratory analyses. In laboratory, the samples were divided into two aliquots: aerated slurry (AS), which was pre-treated by insufflating air for 24 h into the flask at 0.8 L min-1, and untreated slurry (US). Chemical and physical characteristics of liquid fraction were determined on both US and AS samples. Heavy metals (Pb, Cd, Cu, Zn) were determined by atomic absorption spectrometry [13], while ammonia and nitrate [14], phosphate [15], and total polyphenols [16] were determined spectrophotometrically. The organic carbon (C_{org}) was measured as chemical oxygen demand (COD) according to [17], whereas the organic nitrogen (N_{org}) was determined by the Kjeldahl method [18]. The alkalinity was measured by titrating samples with 0.05 M HCl [19].

2.2. Fourier-Transform Infrared (FT-IR) and 1H-NMR Measurements

FT-IR spectra were recorded in modality ATR (attenuated total reflectance) with model Nicolet 5700 by Thermo Electric Corporation (Waltham, MA, USA). The measuring cell consisted of a mono crystal of zinc selenide. The blank was recorded using air as reference. The samples were dried at 115 °C, ground in an agate mortar and recorded in attenuated total reflection mode. ^{1}H-NMR spectra were recorded at 400 MHz in $CDCl_3$ on a Bruker spectrometer (AscendTM400) (Bremen, Germany).

2.3. Microbial Growth and LF Activity

To study the effect of aeration on microbial growth and liquid fraction activity, aliquots of LF were spatulated on plate count agar and incubated for 24 h at 30 °C. Then, the colonies were counted. The LF activity was evaluated as total gas production. In brief, 50 mL of US or AS was put in 100 mL vials and pressure of gases produced inside vials was measured by means of a pressure transducer (Tracker 200). A control vial filled with sterilized US was used too.

2.4. Seed Germination Test, Plant Growth, Photosynthetic Pigments and Total Phenols, and Heavy Metal Mass Fraction

The seeds' germination was evaluated in Petri dish in agar 1.5% and simultaneously also in the soil. Seeds of spinach (*Spinacia oleracea* L. cv Madagascar) were placed in three Petri dishes for treatment, and each contained 30 seeds. The seeds were subjected to three treatments: control (C), in which only 5 mL of $(NH_4)_2SO_4$ solution was added in dishes, untreated slurry (US) or pre-treated slurry (AS) slurry, in which 5 mL of not-aerated slurry or aerated slurry was added, respectively. All Petri dishes were kept in the dark until 15 days at room temperature and germination was monitored and calculated as %.

After germination, 5 plants per treatment were selected for the experiment. The experiment consisted of three different experimental theses: mineral fertilizer (control), untreated (US) and pre-treated (AS) slurry. The germinated spinach seeds were transplanted in pots (0.2 m diameter and 0.15 m depth) filled with sandy-loam soil and irrigated twice a week with 50 mL water. The control plants were fertilized weekly by supplying 29.5 mg N with 50 mL of nutrient solution (N:P:K 7:3:5), the US plants were treated with 29.5 mg N provided by 50 mL of US, and finally the AS plants were fertilized with 12.4 mg N provided by 50 mL of AS. At the end of studied period, plants were collected and the biomass was determined by drying roots and leaves in oven at 75 °C for 48 h. The heavy metal mass fraction in soil, roots and leaves was determined by atomic absorption spectrometry and previous treatment with HNO_3 followed by H_2O_2 in order to destroy the organic matter. Metals' mass fraction was reported as mg kg^{-1} dry weight (DW).

To estimate the root metal uptake from soil and the metal translocation from root to shoot, the bioaccumulation factor (*BF*) and the translocation factor (*TF*) were calculated as [20]:

$$BF = C_i \text{ root or shoot}/C_i \text{ in the soil} \quad (1)$$

$$TF = C_i \text{ shoot}/C_i \text{ root} \quad (2)$$

where C_i is the mass fraction of a single metal.

A concentration index was also calculated, taking into account the high heavy metal content in the soil used in this study, and expressed as:

$$C_i = C_t \text{ US or AS}/C_t \text{ control} \quad (3)$$

where C_t is the total mass fraction of metals in US or AS plants and in control plants.

Photosynthetic pigment (chlorophylls and carotenoids) content was determined according to Lichtenthaler [21] and quantified by a spectrophotometer (UV-VIS Cary 100, Agilent Technologies, Palo Alto, CA, USA) at 470, 645, and 662 nm. Pigment concentrations were expressed in μg cm^{-2}.

Total polyphenols in leaves were determined spectrophotometrically as reported in [22]. The absorbance was measured at 765 nm by a spectrophotometer (UV-VIS Cary 100, Agilent Technologies, Palo Alto, CA, USA). The total polyphenol concentration was calculated and expressed as gallic acid equivalents in mg GAE g^{-1} fresh weight (FW) using a gallic acid standard curve.

Antioxidant content was evaluated as FRAP and determined according to George et al. [23], modified by Costanzo et al. [24]. The absorbance at 593 nm was measured spectrophotometrically (UV-VIS Cary 100, Agilent Technologies, Palo Alto, CA, USA). Total

antioxidants were quantified and expressed as Trolox equivalents in mmol TE g^{-1} FW by means of a Trolox standard curve.

2.5. Soil CO_2 and N_2O Fluxes

The soil CO_2 and N_2O fluxes were carried out on bare soil. PVC pots (0.3 m diameter and 0.10 m depth) were filled with sandy-loam soil and four different treatments were arranged: untreated soil (T), soil irrigated with water (H_2O), soil irrigated with untreated slurry (US), and soil irrigated with pre-treated slutty (AS). In H_2O, US, and AS treatments, 150 mL of liquid was spread on soil.

GHG emissions were measured by the static chamber technique using a 0.2 m wide and 0.1 m height PVC chamber inserted 0.2 m depth into the soil. CO_2 accumulation inside chamber was monitored over 10 min by means of an Infrared Gas Analyzer (CO_2Mini, model RAD-0301, CO_2meter.com) installed inside chamber lid, whereas N_2O accumulation was monitored over 30 min collecting 20 mL air samples at different times by syringe and analyzed by gas chromatography (SRI 8610C Gas Chromatograph, Torrance, CA, USA). The CO_2 measurements were performed 30 min after slurry application, while the N_2O measurements were performed at 3 h, 21 h, and 23 h after slurry application. In the last case (23 h), the measurements were carried out adding a further 150 mL of water.

GHG fluxes were calculated as:

$$F = V/A\ dC/dT \quad (4)$$

where dC/dT is the slope of gas concentration changing over time, V is the chamber volume, and A is the soil surface covered by chamber.

2.6. Statistical Analysis

All data were analyzed using the SigmaPlot 12 software (Jandel Scientific, San Rafael, CA, USA). A one-way analysis of variance (ANOVA) was performed to compare the effect of treatments on chemical and physical characteristics of slurry liquid fraction and seed germination, plant growth, and GHG emissions from soil. Data were treated with Kolmogorov–Smirnov test to check the normality and Tukey's post hoc test was applied for all pairwise multiple comparison tests with a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Effect of Aeration on Chemical–Physical Characteristics of Slurry

The aeration process significantly affected the chemical–physical characteristics of the slurry liquid fraction (LF) (Tables 1 and 2), determining a significant decrease in the heavy metal mass fraction, namely Pb, Cu, and Cd but not Zn (Table 1), and also influencing NH_3, NO_3, total polyphenols (TP), and organic carbon (C_{org}) contents as well as the alkalinity and conductivity (C_{ond}) (Table 2). In particular, a decrease in the NH_4, NO_3, and C_{org} content of 62%, 10%, and 20%, respectively, and an increase in TP and alkalinity of 31% and 84%, respectively, occurred in AS. On the contrary, K, PO4, and organic nitrogen (N_{org}) content was unaffected by treatment.

Table 1. Chemical–physical characteristics of untreated (US) and aerated (AS) slurry. Data are means (n = 5) ± SE.

Treatment	K (g kg^{-1})	NH_3 (mg kg^{-1})	NO_3 (mg kg^{-1})	PO_4 (g kg^{-1})	TP (mg kg^{-1})	Alkalinity (mmol kg^{-1})	C_{org} (%)	N_{org} (mg kg^{-1})	Cond (mS cm^{-1})
US	1.4 ± 0.1 [a]	976 ± 2 [a]	69.3 ± 0.7 [a]	2.6 ± 0.1 [a]	288 ± 3 [a]	50.2 ± 0.7 [a]	0.2 ± 0.1 [a]	14.7 ± 0.7 [a]	7.1 ± 0.1 [a]
AS_{24h}	1.4 ± 0.1 [a]	370 ± 3 [b]	62.8 ± 0.6 [b]	3.0 ± 0.1 [b]	378 ± 2 [b]	92.4 ± 0.8 [b]	0.2 ± 0.1 [a]	14.6 ± 0.8 [a]	7.8 ± 0.1 [b]

K: potassium; NH_3: ammonia; NO_3: nitrate, PO_4: phosphate; TP: total phenols; C_{org}: organic carbon; N_{org}: organic nitrogen; Cond: conductivity. Different letters indicate statistically significant differences among treatments. $p < 0.05$.

Table 2. Heavy metal mass fraction in untreated (US) and aerated (AS) slurry. Data are means (n = 5) ± SE.

Treatment	Pb (mg kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
US	1.7 ± 0.1 ^{a}	1.1 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	3.6 ± 0.1 ^{a}
AS_{24h}	1.4 ± 0.1 ^{b}	0.7 ± 0.1 ^{b}	0.4 ± 0.1 ^{b}	4.8 ± 0.1 ^{b}

Different letters denote statistically significant differences among treatments. $p < 0.05$.

FT-IR analysis of the solid phase identified a large band at 3000 cm^{-1} both in US and AS samples, which was attributed to the intra-molecular and inter-molecular hydrogen-bonded OH groups in phenol, carbohydrate and carboxylic acid compounds [25]. A higher absorbance peak at 3500 cm^{-1}, due to N–H stretching in amines and amides [25], was detected in AS samples compared to US samples, as well as the signal around 2900 cm^{-1} due to the stretching of the C–H bond (Figure 1).

The absorption band centered at 1100 cm^{-1} can be attributed to ether and alcoholic residues (C–O), while the large signal at 1500 cm^{-1} is attributable to the presence of aliphatic compounds with C=C and C–N bonds. The peak around 1700 cm^{-1} is due to the presence of C=O groups (lipids, etc.) [25]. The organic fraction of the solid phase was also studied by ^{1}H-NMR spectroscopy. In particular, an extraction with $CDCl_3$ was carried out on the solid phase of AS and US samples (Figure 2). Aliphatic components are present in relevant concentrations (signals at 1–2 ppm) with respect to the aromatic fraction (around 7 ppm).

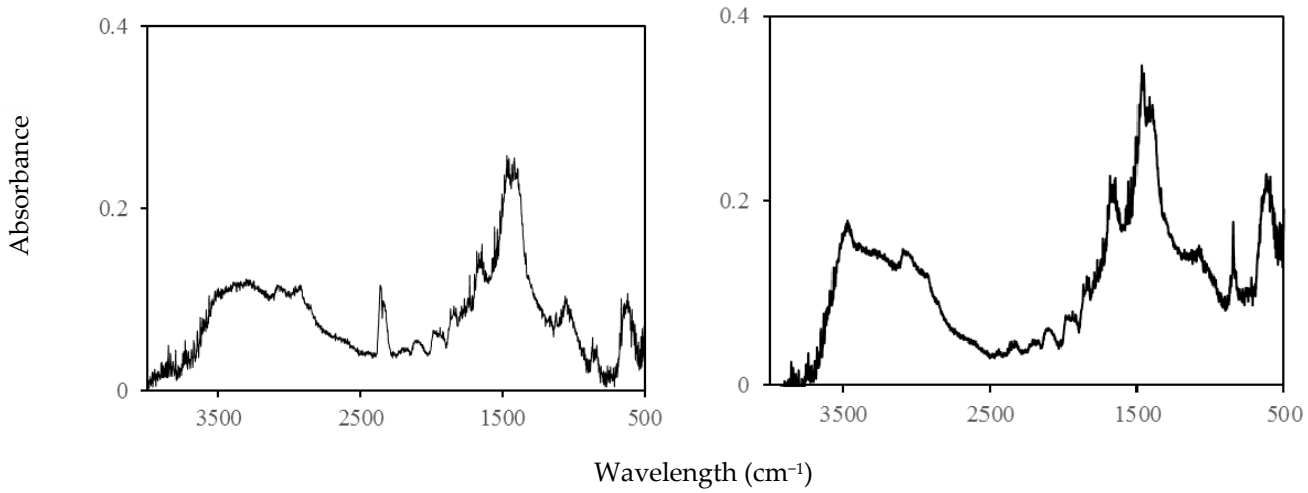

Figure 1. IR spectra from solid phase $CDCl_3$ extract of untreated (US) (**left panel**) and pre-treated (AS) (**right panel**) samples.

Different studies reported the effects of aeration on the chemical–physical characteristics of slurry and on gas loss such as NH_3, CH_4, CO_2, and N_2O during aerobic treatment [26–28]. Indeed, it has been found that the frequency and duration of aerobic treatment, as well as the aeration flow rates, deeply affected the slurry's chemical–physical characteristics, promoting or mitigating gas emissions [26,29–31]. In our experiment, the ammonium content in LF was drastically reduced after 24 h of aerobic insufflation treatment, not likely due to an ammonium oxidation by nitrification that led to N_2O production as the low nitrate content in AS compared to US samples seems to suggest. It may be supposed that the increased alkalinity due to the removal of volatile fatty acids [32] and the bubbling enhanced the N loss as NH_3, while the aeration likely reduced the potential for CH_4 production [29].

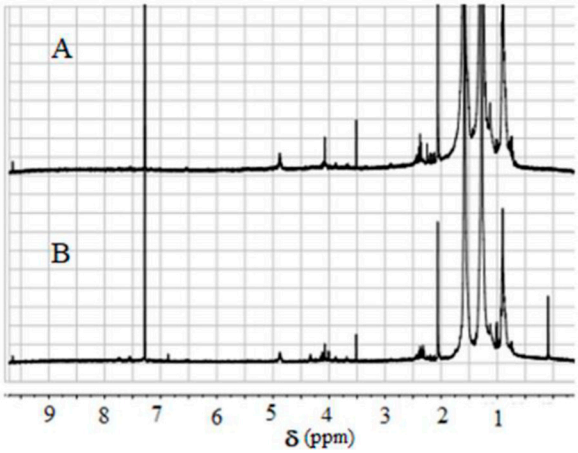

Figure 2. ^{1}H-NMR spectra of solid phase $CDCl_3$ extract of untreated (US) (**A**) and pre-treated (AS) (**B**) samples.

In our study, LF aeration had beneficial effects on the heavy metal mass fraction. We hypothesize that the aeration of slurry determined an increase in pH with the formation of insoluble oxides and hydroxides. The rise in alkalinity might have contributed to reducing the solubility of Pb, Cu, and Cd and enhancing the solubility of Zn [33]. In addition, the alkaline environment might also have increased the surface adsorption of metal cations on silicates and aluminosilicates, contributing to reducing their availability. This result is interesting because slurry is a major source of heavy metal pollution in arable soil. An excess of toxic heavy metals in agricultural soil might derive from the disposal on the soil of animal waste, with important consequences on human health if soils are allocated to food production.

3.2. Effect of Aeration on Microbial Growth and Activity

Figure 3 shows microbial growth for untreated slurry (US) and aerated slurry (AS).

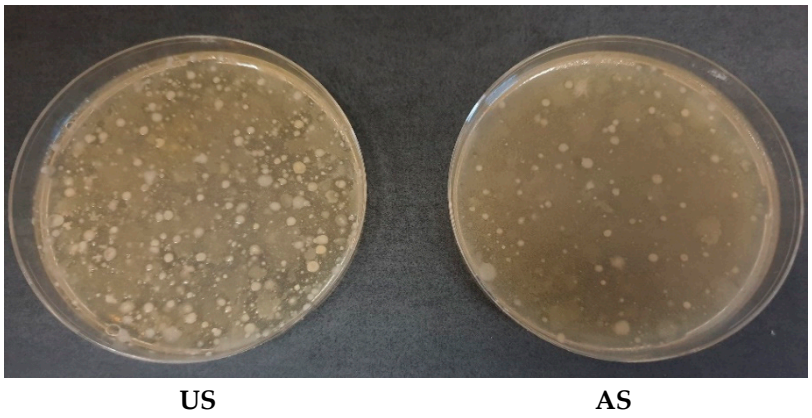

Figure 3. Microbial growth for untreated (**US**) and pre-treated slurry (**AS**).

In US plates, microbial colonies were 400 cfu mL^{-1}, whereas in AS colonies were only 144 cfu m^{-1}. LF activity, evaluated as gas pressure produced in vials, mirrored microbial growth, resulting in 1.003 ± 0.063 psi in US and 0.690 ± 0.058 psi in AS. In control vials, filled with sterilized US, the measured value was 0.703 ± 0.052 psi. Our results show that air insufflation promotes a reduction in microbial growth and in LF activity in terms of gas emissions after treatment. It likely that air insufflating might have favored the growth of aerobic forms to the detriment of anaerobic ones, with a consequent reduction in CH_4 production, while the bubbling might have favored the removal of dissolved NH_3 in LF.

3.3. Seed Germination

To evaluate the effectiveness of aeration on phytotoxic properties of the liquid fraction of slurry, a seed germination test was set up. The untreated (US) slurry seriously inhibited spinach seed germination on agar but not on soil (Table 3), likely due to the buffering capacity of soil. Our results support the previous evidence on the phytotoxicity per se of the slurry deriving from dissolved compounds. Among these, ammonia is known for its phytotoxicity to seeds and seedlings [34] together with other nitrogenous compounds resulting from amino acid metabolism, such as biogenic amines and phenolic compounds [35]. In our experiments, the untreated slurry (US) samples were characterized by the highest ammonia amount, whereas the total polyphenol content was the lowest. These findings suggest that ammonia was likely the main factor that inhibited the germination of spinach seeds even if Cu, present in US in a higher amount than in AS samples (Table 2), may have had a slight inhibiting effect too.

Table 3. Percentage of seed germination. Control ($(NH_4)_2SO_4$), untreated (US) and aerated (AS) slurry. Data are means (n = 3) ± SE.

Treatment	Agar (%)	Soil (%)
Control	44 [a]	50 [a]
US	0 [b]	49 [a]
AS_{24h}	52 [c]	51 [a]

Different letters denote statistically significant differences among treatments. $p < 0.05$.

3.4. Plant Growth and Metabolites, and Plant–Soil Interaction

Soil fertilization with LF promoted the plant biomass of US and AS compared to control plants (Figure 4a). The increase was greater in AS than in US plants due to a greater dry matter allocation to roots than shoots (Figure 4b,c). As a consequence, the shoot/root ratio decreased in AS compared to control and US plants (Figure 4d). We suppose that the greater allocation of dry matter in AS plants may be attributed to the reduced NH_3 content in pre-treated slutty. Ammonia is known to be phytotoxic for plant growth, especially for roots, even at low concentrations [36]. The grater carbon allocation to roots likely improved nutrient and water uptake—if it is considered that AS plants received a lower N content with fertilization than control and US ones (Table 1)—resulting in enhancing nutrient use efficiency by plants. Finally, the antioxidant properties in AS plants improved compared to control and US plants, as indicated by the higher total phenol content and antioxidant activity (FRAP) (Table 4). These results are related to the lower nitrogen content in AS compared to control and US plants, as highlighted by the lower chlorophyll level (Table 4), which represents a proxy for leaf nitrogen content [37]. Recently, Bustamante et al. [38] reported a negative correlation between leaf N and phenols, emphasizing that the phenolic production's response to fertilization was due to leaf nitrogen. Langenkämper and colleagues [39] reported similar results. It is likely that N availability controls the synthesis of the different types of secondary metabolites that, in turn, contribute to improving the leaf antioxidant capacity (FRAP).

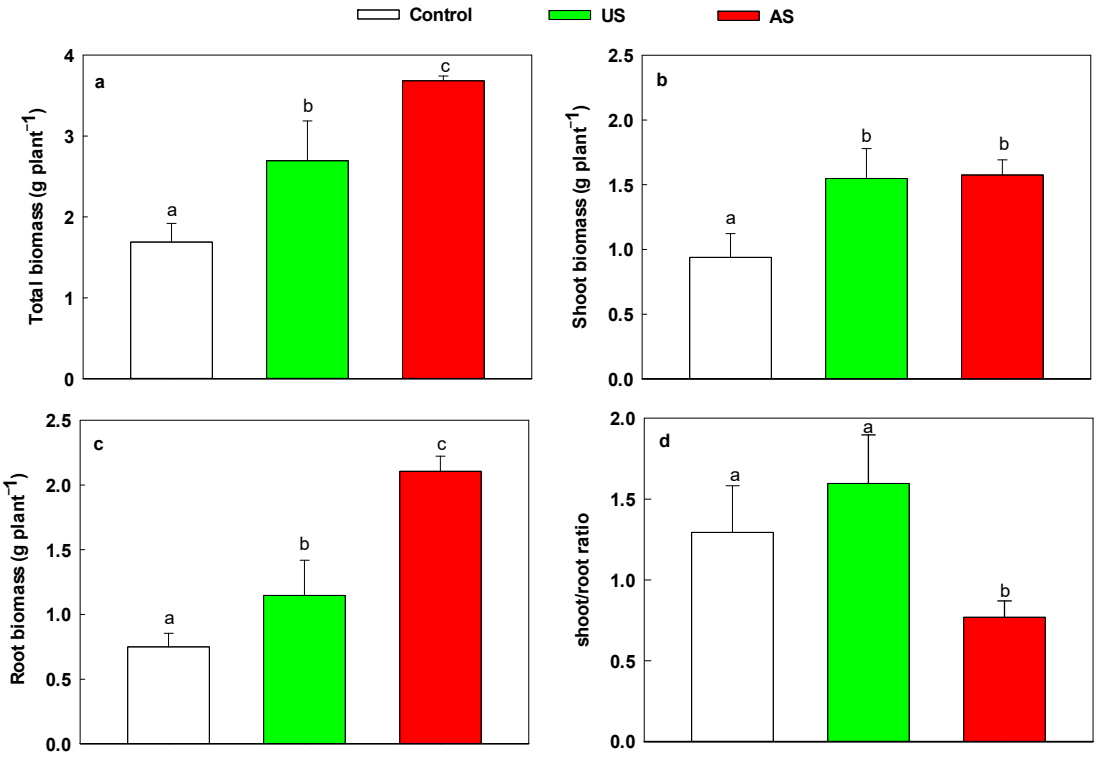

Figure 4. Total biomass (**a**), shoot biomass (**b**), root biomass (**c**), and shoot/root ratio (**d**) of plants fertilized with commercial nutrition solution (control) and in plants fertilized with untreated (US) and pre-treated (AS) slurry. Data are means (n = 5) ± SE. Different letters denote statistically significant differences among treatments. $p < 0.05$.

Table 4. Chlorophyll (Chl), carotenoid (Car), total phenols (Phenols), and antioxidant capacity (FRAP) in control plants and in plants fertilized with untreated (US) and aerated (AS) slurry. Data are means (n = 5) ± SE.

Treatment	Chl (μg cm^{-2})	Car (μg cm^{-2})	Phenols (mg GAE g^{-1} FW)	FRAP (mmol Trolox$_{eq}$ g^{-1} FW)
Control	61 ± 5 ^{a}	10 ± 6 ^{a}	1.8 ± 0.2 ^{a}	479 ± 5 ^{a}
US	53 ± 6 ^{b}	11 ± 1 ^{a}	1.6 ± 0.2 ^{a}	539 ± 14 ^{b}
AS_{24h}	47 ± 5 ^{c}	10 ± 3 ^{a}	2.2 ± 0.1 ^{c}	568 ± 17 ^{c}

Different letters indicate statistically significant differences among treatments. $p < 0.05$.

In the present study, the mass fraction of heavy metals in plants fertilized with the pre-treated slurry (AS) was lower compared to plants fertilized with untreated slurry (US) (Table 5); moreover, the translocation factors (Equation (2)) calculated for Pb, Cd, and Cu were far lower than those measured for US plants, supporting the idea that these metals are sequestered in roots and only slightly moved towards the upper edible plant parts (Table S1). This finding deserves particular attention because slurry spreading on soil is a common agronomic practice carried out by zootechnical farms and it might represent a potential risk for heavy metal accumulation in the soil. The presence of an elevated amount of heavy metals in plants inhibits important enzymatic activities, resulting in adverse effects on seedlings' growth and development [40,41]. It is likely that the lower metal amount in pre-treated slurry (AS) as compared to untreated slurry (US) influenced the mass fraction of heavy metals in the soil, and in turn, their uptake and translocation towards shoots. The concentration index (CI, Equation (3)) in AS plants lower than 1.0 indicated the reduced heavy metal uptake by plants, confirming the above statement (Table S1).

Table 5. Lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn) mass fraction in roots and leaves of control plants and in plants fertilized with untreated (US) and aerated (AS) slurry. Data are means (n = 5) ± SE.

Treatments	Pb (mg kg^{-1} DW)		Cd (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
Control	69.5 ± 1.6 ^{a}	57.8 ± 2.0 ^{a}	29.4 ± 6.1 ^{a}	32.9 ± 3.4 ^{a}	44.4 ± 5.4 ^{a}	12.0 ± 0.3 ^{a}	140.3 ± 11.7 ^{a}	102.2 ± 1.8 ^{a}
US	98.6 ± 5.9 ^{b}	92.0 ± 1.4 ^{b}	17.5 ± 1.4 ^{b}	26.2 ± 3.4 ^{b}	54.3 ± 3.2 ^{b}	15.6 ± 0.8 ^{b}	107.2 ± 3.7 ^{b}	105.1 ± 7.9 ^{a}
AS_{24}	71.0 ± 12.4 $^{a\,b}$	28.1 ± 2.7 ^{c}	16.6 ± 3.4 ^{b}	18.9 ± 3.3 ^{c}	51.8 ± 4.7 ^{b}	8.3 ± 0.3 ^{c}	80.7 ± 14 ^{c}	77.3 ± 2.0 ^{b}

Different letters indicate statistically significant differences among treatments. $p < 0.05$.

3.5. Soil CO_2 and N_2O Emissions

Slurry spreading on arable soils has an impact on greenhouse gas loss, negatively affecting the global climate and environment. For this reason, it is necessary to identify solutions useful to mitigate GHG emissions after slurry spreading on the soil. In the present study, the application of the liquid fraction of slurry on soil determined an increase in CO_2 and N_2O emissions compared to untreated soil and soil treated with H_2O (Figure 5a,b). The liquid fraction of slurry presents labile substances and compounds that are oxidized by soil microorganisms to sustain their growth, whereas LF aeration likely favored a reduction in these substances. As a consequence, the pre-treated liquid fraction of slurry (AS) induced lower CO_2 and N_2O emissions compared to untreated slurry (US) following its disposal on the soil (Figure 5a,b). On the other hand, the spreading of the liquid fraction on soil filled the water pore space to 60%, favoring the nitrification over the denitrification process [42]. Conversely, the lower ammonia content (Table 1), with ammonia being the substrate for oxidizing microorganisms [43], allowed lower N_2O fluxes from AS soils, also when soil was further moistened to simulate rainfall (Figure 4b). Reducing GHG emissions from soil is advantageous for the climate considering that nitrous oxide significantly contributes to climate warming, having a global warming potential 265–298 times that of CO_2 for a 100-yr timescale.

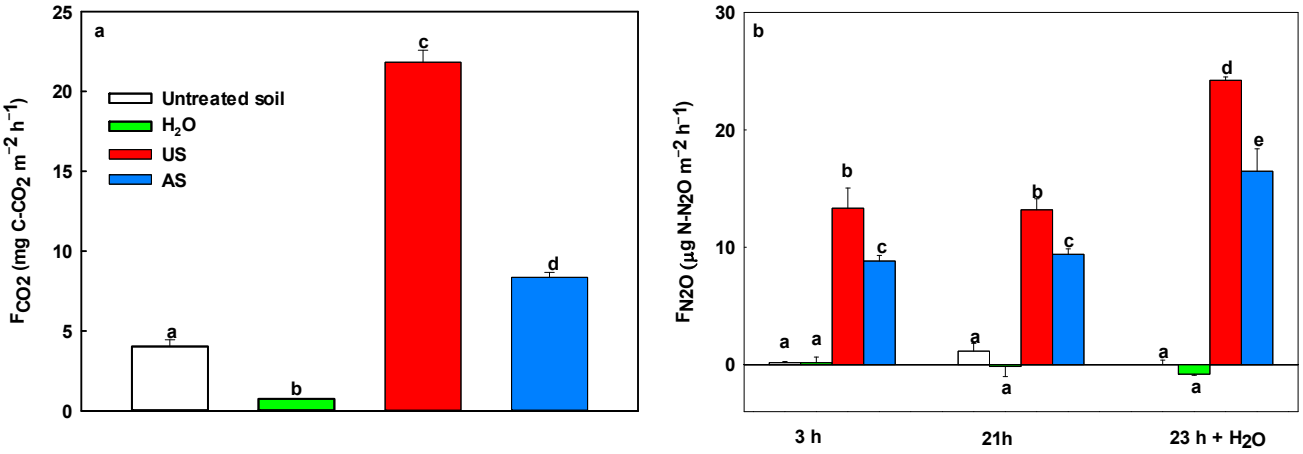

Figure 5. CO_2 (**a**) and N_2O (**b**) fluxes in untreated soil, in soil supplied with H_2O, and untreated (US) and pre-treated (AS) liquid fraction of slurry. Data are means (n = 5) ± SE. Different letters denote statistically significant differences among treatments. $p < 0.05$.

4. Conclusions

Aeration has been shown to be an advantageous practice in order to reduce the heavy metal and ammonia content in the liquid fraction (LF) of buffalo slurry. Once applied to the soil, treated LF proved to be an excellent fertilizer by supporting spinach growth, improving the spinach antioxidant content, and reducing GHG emissions from the soil. The results of this study clearly show that aeration is a valid alternative technique for animal waste management. Although an increase in GHG emissions might occur during LF

treatment, it may be argued that this increase does not necessarily indicate a conflict related to emission mitigation strategy. A possible loss of GHGs during slurry treatment may be mitigated by reduced emissions during subsequent field applications. Our laboratory-scale study suggests that the aeration treatment could represent an alternative and economic method to treat animal wastes, whose employment on a large scale could have positive outcomes in ameliorating crop yields and reducing the environmental impact due to the disposal of great amounts of animal slurry.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11080758/s1, Table S1: Metal bioaccumulation factor in roots (BF_{Root}) and leaves (BF_{Leaves}); translocation factor (TF), and concentration index (CI).

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