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Research Paper Title	Evaluating Acidified Miscanthus Biochar as a Broiler Litter Amendment for Ammonia Control		
M.S. or Ph.D.	M.S.	**Expected Date of Graduation (month/year)**	May 2023

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MAJOR PROFESSOR AND DEPARTMENT HEAD ENDORSEMENTS

I attest that the student named above is a member of ASABE, was enrolled in a graduate program in our department for at least four months between March 15 of this year and March 15 of the previous year and the paper being submitted is based on research completed for either M.S. or Ph.D. degree.

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Evaluating Acidified Miscanthus Biochar as a Broiler Litter Amendment for Ammonia Control

Highlights

- Biochar pyrolysis temperature, acid modification selection, and application rate were statistically significant ($p < 0.01$) for ammonia emissions.
- A stronger acid (citric acid) did not provide more effective ammonia reduction compared to a weaker acid (acetic acid).
- Pyrolysis temperatures of 400°C were better at retaining oxygen functional groups and had higher acidity.
- Residual acid washing contributed to the decreased performance of biochars compared to sodium bisulfate.

Abstract. *Biochar from lignocellulosic biomass has proven to be a versatile tool in environmental remediation applications for water, soil, and air quality. This study investigated miscanthus biochar potential to reduce ammonia (NH_3) emissions associated with poultry production. NH_3 emissions present a concern for animal and human health and the environment. Utilizing biochar to address this challenge creates a unique synergy between biomass/biofuels and food animal sectors. Optimizing the physicochemical properties of the biochar can enhance its adsorption capacity. The goal of this study was to test the impacts of biochar production temperature, organic acid activation, and application rate on its performance as a broiler litter amendment to reduce NH_3 emissions. A randomized block experiment evaluated biochar produced at 400 and 700°C, activated with acetic or citric acid and applied at two addition rates to the litter: low (0.24 kg m^{-2}) and high (0.49 kg m^{-2}). Biochar production parameters, i.e., temperature, and acid type, significantly affected its performance for NH_3 control. Ordered by magnitude, the following factors statistically influenced NH_3 emission rate: biochar application rate ($p < 0.001$), biochar production temperature ($p = 0.003$), and lastly acid type ($p = 0.007$). The best performing biochar was produced at 400°C, activated with acetic acid, and applied at a high addition rate (0.49 kg m^{-2}). This treatment reduced cumulative NH_3 volatilization after two weeks by 19.7%. As a reference, the positive control, sodium bisulfate, reduced NH_3 by 92.2% after two weeks. Future work should focus on larger scale trials and using different acidification methods to optimize carboxyl and other acidic groups on the biochar surface.*

Keywords. *Acidified biochar, agricultural emissions, ammonia mitigation, litter amendment.*

INTRODUCTION

As the global population is expected to reach 9.8 billion by 2050, there is an increasing need to ensure food security to sustain this growth (Searchinger et al., 2018). Urbanization is changing the way people buy and consume food resulting in an increase in large scale animal production (Henchion et al., 2017). Projections for protein demand are of particular interest, with the FAO estimating the demand for animal-derived protein to increase by 102% by 2050 (Boland et al., 2013; Alexandratos & Bruinsma, 2012). Poultry leads the globe in meat consumption, ahead of pork and beef, and is expected to continue increasing at an annual rate of 2% through at least 2031. Poultry protein is preferred due to its convenience, consistent product quality, low fat content, low cost of production, and consumer affordability (Kleyn & Ciacciariello, 2021; Dohlman et al., 2022).

Addressing global food security by improving poultry production efficiency is a priority. Similar to other confined animal feeding operations (CAFOs), high animal density increases concerns for air, soil, and water quality inside and near these operations (Sharpley, 1998; Burkholder, et al., 2007). Most notable for poultry production is the high levels of ammonia (NH_3) inside the barns, that are released into the atmosphere. This ammonia is produced by the decomposition of uric acid in the feces (Ferguson et al., 1998; Emous et al., 2019). The EPA estimates that 0.20 kg NH_3 is emitted per bird placed per year. With the US alone producing 9.3 billion finished birds per year, this becomes a significant environmental impact (Baker et al., 2019; USDA, 2022).

Ammonia volatilization from poultry production presents several concerns for animal health and welfare, human health, and the environment (Shah et al., 2012). As NH_3 concentrations increase, there is increased risk of bird diseases, including footpad dermatitis, reducing overall productivity (Shepherd & Fairchild, 2010; Kaukonen et al., 2016). At low concentrations humans can experience eye and throat irritation and the odor produced have significant quality of life impacts for workers and surrounding communities (Sundblad et al., 2004; Blanes-Vidal et al., 2012). Ammonia released into the atmosphere can deposit and result in harmful nutrient imbalances in soil and water systems

(Longo et al., 2021). With increases in bedding cost, litter is reused for multiple flocks, only exacerbating the problem (Diarra et al., 2021). For these reasons, researchers and growers prioritize NH_3 emission reduction at the source in poultry houses.

Chemical and biological transformations are the driver for NH_3 volatilization. Microorganisms within the litter drive the decomposition of the manure, transforming organic nitrogen (N) and uric acid in the manure into ammonium (NH_4^+) which can volatilize as NH_3 (Jensen & Sommer, 2013). Simultaneously, the impact of temperature, pH, and moisture on the NH_4^+/NH_3 equilibrium reaction has a large impact on the volatilization potential (Du Plessis & Kroontje, 1964). Strategies to control NH_3 emissions include changes to animal diet, controlling house humidity levels and temperature, and utilizing additives or amendments to manipulate manure pH and microbial activity (Zhao et al., 2014). A major class of litter amendments include acidifying agents, like sodium bisulfate, aluminum sulfate or alum, and clay treated with sulfuric acid. All of these agents work by lowering the pH of the litter into an acidic range to trap NH_4^+ within the litter and reduce volatilization (Joerger et al., 2020; Choi & Moore, 2008). Alum has been shown to reduce NH_3 emissions from poultry litter by 70% for three weeks after application, while sodium bisulfate reduces NH_3 by 90% for two weeks after application (Tasistro et al., 2007). Some of these agents however are found to have corrosive properties, requiring protection of fans and concrete block walls (Hunolt et al., 2015). Another class of litter amendments include adsorbers, which reduces NH_3 volatilization by physical means rather than chemical. These typically include naturally occurring, porous biomaterials like zeolite, bentonite, and peat (Wlazło et al., 2016).

Recent research has explored biochar, as a litter amendment due to its adsorbent properties (Linhoss et al., 2019; Ritz et al., 2011; Flores et al, 2021; Doydora et al., 2011). Biochar is a carbon rich residue produced by the high temperature treatment (300-900°C) of biomass in the absence of oxygen (i.e., pyrolysis). During this process, volatile organic compounds are released from the biomass particle increasing its surface area, at times to several thousand times that of the original particle (Beesley et al., 2011). Additionally, biochar properties can be further modified via physical and chemical methods to increase the surface area or add specific functional groups to the surface. Biochar continues to be investigated for various applications, including the adsorption of NH_4^+ (Ro et al., 2015; Salimova et al., 2020).

Linhoss et al. (2019) found the addition of biochar to pine shaving poultry bedding increased water holding capacity by up to 32.2% and reported no negative effects on bird health. Flores et al. (2021) had similar results, finding no negative impacts on bird health as well as high body weight at 20 weeks. Ritz et al. (2011), on the other hand, found that peanut hull biochar addition alone was not enough to significantly reduce litter NH_3 emissions. Alternatively, they also evaluated acidified pine bark and coconut husk biochar activated with sulfuric acid which reduced NH_3 emissions by 440 mmol NH_3 kg^{-1} m^{-2}, close to 50%. This acidification without subsequent washing lowered the amendment pH from 9.2 to 2.0. In a slightly different application Doydora et al. (2011) found that land applied poultry litter treated with HCl activated pine chip and peanut hull biochar (both pH 2.50) reduced NH_3 volatilization by 58-63%. Most recently, Baral et al. (2023) treated solid separated anaerobic digestate with orthophosphoric acid activated Miscanthus biochar and digestate biochar. The acid activation, without washing, lowered the pH of the Miscanthus biochar from 10.4 to 4.8 while the digestate pH remained 8.9. The treatments reduced NH_3 emissions by 37-51% in the first month of storage.

These studies demonstrate that acid activated biochars, without a washing step, are more effective in NH_3 emission control than non-acidified biochars. However, there still remain many research questions on what physical and chemical properties contribute to the greatest NH_3 adsorption potential. In this study, biochar derived from miscanthus grass (*Miscanthus* × *giganteus*), a common fast-growing bioenergy crop originating in Asia, are applied to fresh broiler litter to compare biochar production temperatures, acid types, and acid strength on the reduction of NH_3 volatilization. The purpose of this study is to explore the potential for a litter amendment combining adsorbent properties and milder acidifying properties to control ammonia emissions and reduce the use of corrosive acids inside poultry houses and promote green chemistry practices for environmental remediation.

MATERIALS AND METHODS

2.1 LITTER COLLECTION AND CHARACTERIZATION

Broiler litter was collected from a commercial broiler farm in Randolph County, North Carolina, USA. The farm is under contracted broiler production with Mountaire Farms (headquartered in Little Rock, AR). Litter was collected at 6 weeks into a flock with 12 flocks previously raised on this litter (2.5 year old litter). Wood shavings were used as bedding material and litter had received sodium bisulfate treatment at the beginning of the flock. Sampled litter was collected from multiple spots throughout the house, excluding wet areas near the watering lines and feeders. A 15 kg sample of litter was collected and transferred to the BAE Department laboratory at NC State University and cold stored (at 4°C). The triplicate samples of the collected litter were analyzed Agronomic Services Lab (North Carolina Department of Agriculture and Consumer Services, Raleigh, NC) to quantify total N, NH_4-N, NO_3-N, total C, C:N, MC%, and pH).

2.2 BIOCHAR PRODUCTION

Miscanthus grass (*Miscanthus x giganteus*) grown in Goldsboro, NC was provided by AGgrow Tech as the biomass feedstock for biochar production. The Miscanthus was heated in a muffle furnace to 400 or 700°C in the absence of oxygen, i.e. pyrolyzed, for 4 h using nitrogen as the carrier gas. Thereafter, the resulting biochar was ground to a uniform particle size of 0.1 mm and subject to acid activation following methods by Doyadora et al. (2011). Acetic acid (AA) and citric acid (CA) were used for activation at 2 mol L^{-1} concentrations for activation.

Composition and electronic state of the biochar samples was analyzed by X-ray photoelectron spectroscopy (XPS) using a SPECS System with PHOIBOS 150 Analyzer. Data reduction, energy calibration, and peak fitting was processed using XPSPEAK41. Additionally, pH and titration methods were used to quantify free hydrogen ions as well as the total acidity present on the biochar using methods described by Gomes et al. (2010).

2.3 Litter Treatments

This study utilizes biochar produced as described above. The effects of biochar production temperature, acid treatment, and application rate on litter ammonia emissions were investigated in this study. In addition to the biochar amended litter, positive and negative control treatments were chosen to benchmark biochar performance. The positive control was sodium bisulfate, (PLT®, Jones Hamilton Co., Richburg, South Carolina, USA), which is a commonly used acidifying product to control ammonia levels (Hunolt et al., 2015). Sodium bisulfate was applied at the equivalent rate as industry recommendations, 0.49 kg m^{-2} (0.1 lb ft^{-2}) (Jones-Hamilton, 2010). The negative control was unamended litter.

Two biochar application rates were selected and coded as a low and high addition to the litter. The low addition rate (L) was set to half the recommended sodium bisulfate rate, 0.24 kg m^{-2} (0.05 lb ft^{-2}) while the high addition rate (H) was equal to the sodium bisulfate recommendation of 0.49 kg m-2 (0.1 lb ft^{-2}). Table 1 lists all the treatments and their application rates tested in this study.

Table 1. Litter amendment treatments used in experiment

Treatment ID	Biochar Temp. (ºC)	Biochar Acid Type	Application Rate (kg m^{-2})	Application Rate (lb ft^{-2})
C	--	--	--	--
PLT	--	--	0.49	0.1
400 - L	400	--	0.24	0.05
400 - H	400	--	0.49	0.1
700 - L	700	--	0.24	0.05
700 - H	700	--	0.49	0.1
400 AA - L	400	Acetic Acid	0.24	0.05
400 AA - H	400	Acetic Acid	0.49	0.1
700 AA - L	700	Acetic Acid	0.24	0.05
700 AA - H	700	Acetic Acid	0.49	0.1
400 CA - L	400	Citric Acid	0.24	0.05
400 CA - H	400	Citric Acid	0.49	0.1
700 CA - L	700	Citric Acid	0.24	0.05
700 CA - H	700	Citric Acid	0.49	0.1

2.4 Experimental Set-up

A set of temperature-controlled volatilization chambers, previously used by Kulesza et al. (2014), and originally designed and validated by Woodward et al. (2011) were used in this study as shown in Figure 1. Each volatilization chamber is a glass, threaded-top jar 100 mm (3.94 in) diameter, 150 mm (5.91 in) height, with airflow fittings in the cap. Each chamber was filled with 186 g (0.41 lb) of as-received broiler litter, for a target depth of 50 mm (1.97 in), then covered with the selected amendment. Figure 1 illustrates the experimental setup used to test the impact of different amendments on litter emissions. Each treatment was evaluated in four replicates, with chambers maintained at 25°C (77°F) throughout the testing period (14 days). Upstream to the chambers was an air pump, flowmeter, and two humidistats to maintain incoming air 100% humidity to increase the response variable (Cassity-Duffey et al., 2015). Downstream of the litter chambers were bottles filled with 200 ml 0.04 M phosphoric acid (H_3PO_4) to trap gaseous NH_3 released during the trial (Hunolt, 2015). A Hiblow® 80A Septic Air Pump provided airflow with flow rates controlled using Omega™ acrylic mechanical flow meters set to 1 L min^{-1} (0.035 ft^3 min^{-1}). Flowmeters were calibrated at the beginning of the experiment using an Omega™ FMA 1818A mass flow meter. A timer (24 Hour BN-LINK) was utilized to control pump operation using a 5 minute on, 30 minute off cycle to maintain an air exchange rate (ACH) of 15 h^{-1} to mimic air exchange rates of a typical broiler house (Carr et al., 1990). Each run was conducted for a total of two weeks. During each run, treatments were simultaneously evaluated in four replicates, each with a positive and negative control.

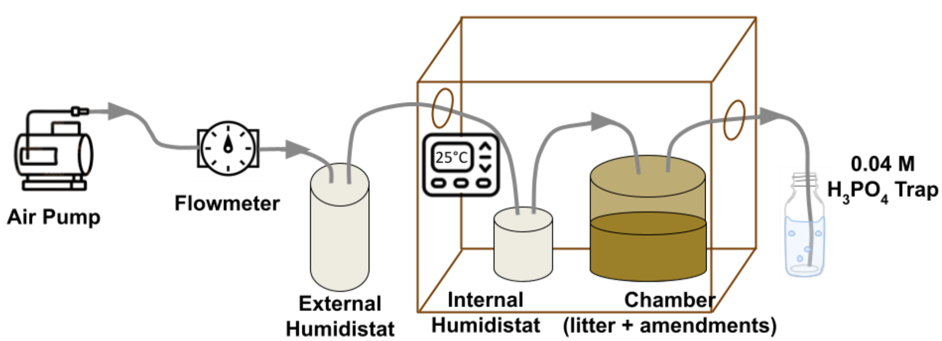

Figure 1. Ammonia volatilization study chamber set-up

2.5 Ammonia Emission Quantification

Acid traps were replaced at the following intervals (in hours from the test start): 1, 3, 6, 9, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 hours.

Collected acid bottles were capped and immediately refrigerated at 3°C (38°F) until analysis. To track the mass balance of the system, humidistats and litter chambers were weighed at the start and end of the study, as well as all acid bottles before and after placement. The entire system was routinely checked to verify flow rates, the timer setting, and acid traps were in place and bubbling exhaust airflow as expected. A 50 ml aliquot from each used acid bottle was submitted to the Environmental Analysis Lab (NC State University) for quantifying trapped NH_3. A K_2SO_4-$CuSO_4$ digestion was conducted prior to ammonia-salicylate-nitroprusside-hypochlorite calorimetry analysis on a Lachat Instrument Autoanalyzer System. A dilution factor of 100 was applied to all samples.

2.6 Statistical Analysis

Treatments in this study were arranged in a randomized block design with each chamber representing an experimental unit while each box is an experimental block. Student t tests were conducted for a means comparison between amendment properties and cumulative NH_3 release after one and two weeks using JMP Pro 16 statistical package (SAS Institute, 2019). Statements of statistical significance were accepted at $\alpha < 0.05$.

Results and Discussion

3.1 Biochar Characteristics

Acidified and unmodified biochar properties are included in Table 2. Increasing pyrolysis temperature from 400 to 700°C increased biochar carbon content by 7% as a result of a greater volatile matter loss due to higher conversion severity (Li et al., 2017). Acid modification using citric or acetic acid had no effect on total oxygen (O). Increased biochar production temperatures led to increases in biochar pH from 7.29 to 9.95. Higher pyrolysis temperatures resulted in an increase in pH due to the decrease of organic functional groups such as carboxyl and hydroxyls (Chellappan et al., 2018).

Acidified biochar had significantly lower pH levels between 2.71 and 3.88. Citric acid treated biochars had significantly lower pH levels than acetic acid treated chars for both temperature levels, attributed to the fact that citric acid has three carboxyl groups and acetic acid has one. Acidified biochars pH was comparable to values observed in commercial litter amendments, e.g., aluminum sulfate (alum) and acidified clay have a pH < 3, putting them in the desirable range as an acidic litter amendment. Acidity values shown in represent the combined effect of the following groups: carbonyl, carboxyl, hydroxyl, and lactone groups. Total acidity was higher in biochar produced at 400°C than 700°C, with a mean of 968 and 477 μmoles/g acidic group, respectively. This trend was expected considering O groups contribute greatly to acidity and higher pyrolysis temperatures observed a reduction in O content (Wang et al., 2020). Additionally, acid activation increased total acidity by 33-73% in 700°C biochar and 80-88% in 400°C biochar.

Acid activation aims to transfer acid groups (-COOH) to the biochar surface, and acidification methods seem to play a large role in this transfer. The biochar and acid mixing duration used by Doydora et al. (2011) was a short duration compared to other studies who used mixing durations of 3 to 24 hours (Liu et al., 2020). This observed phenomenon could also be attributed to the acid type used in this study, i.e. citric and acetic aids, which are considerably weaker than mineral acids such as nitric, phosphoric, and sulfuric acids. Current studies that evaluate acetic or citric acid modified biochar do not report acidity values, only pH (Sun et al., 2015). Another important factor in the attachment and presence of acid groups is whether a residual acid washing step was used. Several studies do not report washing the acidified biochar prior to testing. Meanwhile, others report washing the biochar with water until the biochar reaches a neutral pH of 7.0 (Lonappan et al., 2020). Unwashed, acidified biochar will show greater acidity values due to acid residuals present. The lack of studies solely on post-activation processing (i.e. washing, acidification duration) makes it difficult to conclude any of these reasons alone are responsible for the results found in this study.

Table 2. Chemical properties of Miscanthus biochar before and after activation using acetic acid (AA) and citric acid (CA)

Biochar Type	400°C	400°C - AA	400°C - CA	700°C	700°C - AA	700°C - CA
Mass Yield (%)	34.1%	-	-	24.5%	-	-
Carbon (wt. %)	85%	84%	84%	92%	95%	95%
Oxygen (wt. %)	15%	16%	16%	8%	5%	5%
pH	7.29 ± 0.04	3.88 ± 0.30	2.71 ± 0.12	9.95 ± 0.13	3.54 ± 0.09	3.08 ± 0.06
Acidity (μmoles/g)	968 ± 66	1,739 ± 56	1,821 ± 22	477 ± 96	636 ± 46	826 ± 110

3.2 Litter Characteristics

Litter properties before and after emission testing are shown in Table 3. Initial litter samples did not contain any amendments, while final samples included respective amendments, except for the negative control (C) samples. Consistent trends observed among all treatments include an increase in litter moisture content from 6.6-13.5% due to. A decrease in total nitrogen, ranging from 4.6-9.8% (dry basis) was observed for all biochar treatments after two weeks of incubation, while the control saw a decrease of 6.2% and sodium bisulfate 3.7%. This decrease is attributed mostly to NH_3 volatilization, which was captured in the acid traps throughout the experiment. Mineralization of N from organic to non-organic forms (i.e. NO_3 and NH_4) and nitrification and denitrification processes are potential pathways for N loss. Nitrification takes approximately two to six weeks, so this pathway is less likely to have played a large role in N loss from litter samples. Finally, a decrease in pH by 1.7% in the control, 4.6% in sodium bisulfate, and 0.5-1.8% for all biochar treatments. The reduction in pH for the control is attributed to the organic matter decomposition similar to all other treatments, as well as the increased NH_3 volatilization since ammonia is a basic compound. The combination of these three properties, i.e., moisture content, pH, and nitrogen content and speciation, are the largest factors contributing to NH_3 formation of in poultry litter (Ritz et al., 2004). In general, NH_3 emissions are higher when litter pH is above 8.0, while litter between 7.5 and 8.5 will show 50-80% of total available N in the litter converted to NH_3 (Carr et al., 1990; Reece et al., 1979).

Table 3. Broiler litter properties before and after ammonia volatilization testing (two week duration)

	Treatment ID	Moisture Content (%)	Total N ($g\ kg^{-1}$)[a]	NH_4-N ($g\ kg^{-1}$)[a]	NO_3-N ($mg\ kg^{-1}$)[a]	pH
		Mean ± Std. Deviation				
Initial	--	24.3 ± 0.65	30.4 ± 1.2	5.0 ± 0.2	74.5 ± 7.7	8.26 ± 0.05
Final	C	27.0 ± 0.6	27.5 ± 0.8	4.5 ± 0.2	67.1 ± 12.3	8.12 ± 0.08
	PLT	27.6 ± 1.3	28.0 ± 0.6	5.3 ± 0.8	71.2 ± 9.3	7.88 ± 0.07
	400 - L	26.6 ± 0.2	27.1 ± 0.4	4.7 ± 0.1	62.8 ± 6.1	8.15 ± 0.03
	400 - H	25.9 ± 0.2	28.4 ± 0.2	4.6 ± 0.0	72.3 ± 6.3	8.18 ± 0.02
	700 - L	27.1 ± 0.4	26.4 ± 0.6	4.6 ± 0.2	43.7 ± 8.9	8.13 ± 0.05
	700 - H	26.2 ± 0.3	28.0 ± 1.1	4.6 ± 0.2	73.7 ± 8.4	8.21 ± 0.01
	400 AA - L	26.6 ± 0.1	27.0 ± 0.2	4.7 ± 0.2	63.5 ± 10.2	8.12 ± 0.05
	400 AA - H	26.1 ± 0.4	27.5 ± 0.9	4.5 ± 0.2	70.9 ± 4.1	8.19 ± 0.02
	700 AA - L	26.9 ± 0.6	27.0 ± 0.7	4.7 ± 0.2	58.9 ± 20.2	8.11 ± 0.04
	700 AA - H	26.2 ± 0.1	28.0 ± 0.5	4.6 ± 0.2	72.9 ± 6.1	8.19 ± 0.03
	400 CA - L	27.0 ± 0.2	27.0 ± 1.1	4.3 ± 0.2	81.5 ± 2.6	8.13 ± 0.10
	400 CA - H	27.2 ± 0.3	27.9 ± 0.8	4.3 ± 0.1	76.3 ± 1.9	8.22 ± 0.03
	700 CA - L	27.3 ± 0.4	26.8 ± 0.9	4.3 ± 0.1	83.7 ± 5.8	8.13 ± 0.11
	700 CA - H	27.1 ± 0.4	26.6 ± 1.0	4.3 ± 0.1	78.5 ± 6.9	8.16 ± 0.08

[a] Wet basis

Despite no mechanical mixing after amendment addition (Figure 2a), biochar covers migrated downward through the litter layer (Figure 2b). Vibration, attributed to mechanical fan and air flow inside environment-control boxes, combined with the small biochar particle size (0.1 mm) can be the cause behind this observation. Covali et al. (2021) found that acid modified biochar were less hydrophobic than unmodified chars allowing them to incorporate more when surface applied to cattle digestate. Mixing of amendments and litter would be expected when used in full-scale broiler houses due to bird activity. Mold formation was observed in some experimental units (Figure 3) but there was no specific treatment associated with this observation. The bedding material age (2.5 years), coupled with high moisture level in the incubation chambers are the likely causes of this phenomenon (Bernhart & Fasina, 2008). No fungicides were applied to the litter prior to sampling for our study.

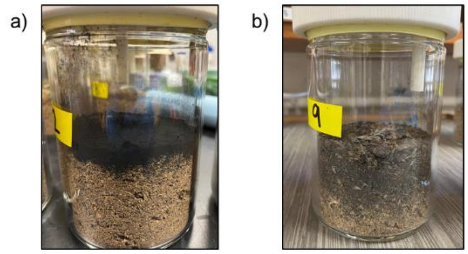

Figure 2. Visual differences in biochar mixing across the litter profile after 2 weeks; some jars showed little mixing (a), while others mixed further down into the litter (b)

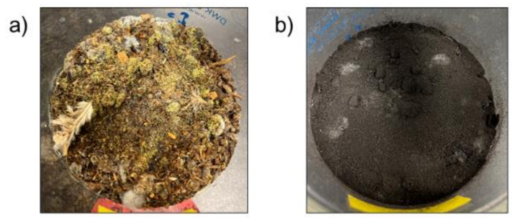

Figure 3. Molding incidents in sodium bisulfate (a) and biochar (b) treatments

3.3 Cumulative Ammonia Emissions

All treatments showed a linear increase in cumulative NH_3-N released throughout the experiment, with R^2 values for linear fit ranging between 0.96-1.00 for all treatments except sodium bisulfate with $R^2 = 0.47$. There were close to zero emissions at the one-week mark for the sodium bisulfate treatment, with a slight decrease in performance between one and two weeks as shown by the second order graph shape. The negative control treatment showed the largest NH_3-N release compared to all other treatments.

Table 4 summarizes cumulative NH_3 released after one and two weeks of incubation. Sodium bisulfate performed significantly better than biochar treatments, with a 92.2% NH_3 reduction at two weeks. Alternatively, the best performing biochar treatment, 400 AA - H, showed a 19.7 % reduction after two weeks. Doyadora et al. (2011) found a 58-63% NH_3 reduction from surface application of acidified biochar and poultry litter to soil. However, biochar and poultry litter were mixed thoroughly at a 1:1 ratio, biochars were treated with HCl for 24 hours, residual acid was not washed away, and the final biochar pH was 2.5. For all these reasons, a much higher reduction in NH_3 emissions can be attributed to the difference in acid strength, residual acid from treatment, and treatment duration. Ritz et al. (2011) had more comparable findings to the present study at an identical application rate of 0.24 kg m^{-2} using biochar acidified using H_2SO_4 without residual acid washing (biochar pH of 2). At application rates of 0.73 kg m^{-2} Ritz et al. found NH_3 reduction increased from 5% to almost 50%.

Table 4. Cumulative NH_3-N released (mg g $litter^{-1}$) during litter incubation

Treatment ID	After one week[a]	After two weeks[a]
C	1.03 ± 0.06 [a]	1.88 ± 0.09 [a]
PLT	0.01 ± 0.01 [h]	0.15 ± 0.09 [g]
400 - L	0.95 ± 0.04 [b, c, d]	1.80 ± 0.08 [a, b]
400 - H	0.84 ± 0.04 [e, f]	1.61 ± 0.06 [e, f]
700 - L	0.91 ± 0.05 [c, d, e]	1.74 ± 0.07 [b, c, d]
700 - H	0.84 ± 0.01 [e, f]	1.63 ± 0.03 [d, e, f]
400 AA - L	0.79 ± 0.13 [f, g]	1.59 ± 0.21 [e, f]
400 AA - H	0.74 ± 0.05 [g]	1.51 ± 0.05 [f]
700 AA - L	0.91 ± 0.06 [c, d, e]	1.73 ± 0.09 [b, c, d]
700 AA - H	0.88 ± 0.05 [d, e]	1.66 ± 0.05 [c, d, e]
400 CA - L	0.96 ± 0.07 [b, c]	1.76 ± 0.09 [b, c]
400 CA - H	0.83 ± 0.03 [e, f]	1.59 ± 0.03 [e, f]
700 CA - L	1.02 ± 0.04 [a, b]	1.86 ± 0.06 [a, b]
700 CA - H	0.94 ± 0.06 [c, d]	1.74 ± 0.10 [b, c, d]

[a] Levels not connected by the same letter are significantly different ($\alpha < 0.05$). Emissions at one and two weeks were run in a fit of least squares, separately.

3.4 Biochar Treatment Impacts on Ammonia Emissions

Analysis of variance was conducted to assess the impact of biochar properties (production temperature, acid type, and application rate) on cumulative NH_3-N released (mg g $litter^{-1}$). An interaction term was added to explore any potential synergies between biochar production temperature and acid type used in activation. Overall, the model goodness-of-fit, $R^2 = 0.50$, indicated biochar described half of the variability observed in emissions.

Biochar amendment rate was by far the most influential factor on NH_3 emissions ($p < 0.001$). Biochar applied at the 0.49 kg m^{-2} rate released an average 1.62 mg NH_3-N g $litter^{-1}$ while the 0.24 kg m^{-2} addition rate released an average of 1.75 mg NH_3-N g $litter^{-1}$. Biochar production temperature was the second most influential factor impacting cumulative NH_3 emissions ($p = 0.003$), with 400°C biochar amended litter releasing 1.64 mg NH_3-N g litter^{-1} and 700°C releasing 1.73 mg NH_3-N g litter^{-1}. Least important, but still statistically significant, was the acid choice (p = 0.007), with acetic acid activated biochar treatments releasing 1.62 mg NH_3-N g litter^{-1} while citric acid biochar treatments releasing 1.73 mg NH_3-N g litter^{-1}.

The performance of the high addition rate versus the low addition rate is possibly due to a thicker cover on top of the litter acting as a physical barrier reducing volatilization. Further analysis of the biochar after the experiment with XPS could have confirmed or rejected this theory. Biochar produced at 400°C was more effective in reducing NH_3 emissions than that produced at 700°C. This observation could be attributed to the higher concentration of oxygen functional groups present (associated with more acidic groups and a lower pH). Acetic acid was significantly better as an activation acid than both unacidified biochar and citric acid at reducing NH_3 volatilization. Although citric acid performed slightly better than no acid addition, there was no statistically significant difference between the two based on a means comparison (1.73 versus 1.69 mg NH_3-N g litter^{-1}). Citric acid resulted in biochar with lower pH than acetic acid, as well as higher acidity so it cannot be determined whether pH or acidity are the greatest contributor. Although citric acid is the stronger acid, the biochar was washed after acid activation removing residual acid from the biochar surface possibly minimizing this effect (Soto-Herranz et al., 2022).

The assumption would be that the acetic acid was then more effective at transferring acidic groups to the biochar than citric acid due to its increased performance, but that is not supported by the titration data in Table 1. Previously, Lonappan et al. (2019) found that citric acid at comparable strengths increased total acidic functional groups by 10-26% in pinewood, pig manure, and almond shell biochars. Their study soaked the biochar in acid for 24 hours which could be a large contribution to their more successful activation.

CONCLUSIONS

Two week litter incubation studies confirmed that biochar application rate is the most important factor for a litter amendment, followed by the biochar production temperature, and lastly acid type. The presence of acidic sites on the biochar had a strong correlation to increased NH_3 reduction. The most effective treatment was 400 AA - H, which was biochar produced at 400°C, treated with acetic acid and applied at a high addition rate with a 19.7% NH_3 reduction after two weeks. A commonly used product in the poultry industry, sodium bisulfate, saw 92.2% reduction after two weeks. While the goal of this study was not to compare acidified biochar performance to products currently used in commercial applications, it is a benchmark of how much improvement needs to occur for biochar to be considered a viable alternative in the poultry industry and provide a return on investment. Biochar activation methods are essential to adequately modifying functional groups and pH of the biochar. Longer soaking times and no residual acid washing step increase successful activation.

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