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USE OF BEDDING MATERIALS IN BEEF BEDDED MANURE PACKS IN HOT AND COOL AMBIENT TEMPERATURES: EFFECTS ON AMMONIA, HYDROGEN SULFIDE, AND GREENHOUSE GAS EMISSIONS



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HIGHLIGHTS

- Lab-scale beef manure bedded packs were constructed to evaluate the temperature effect on gaseous emissions.
- Temperature had a tendency to increase ammonia, hydrogen sulfide, and greenhouse gases in the headspace above bedded packs over time.

ABSTRACT. Throughout the Upper Midwest, producers have observed increased land and fertilizer prices, resulting in increased popularity of confinement feeding facilities such as mono-slope and hoop barns with bedded packs. Environmental and public pressure has been placed on the agriculture community to reduce ammonia (NH_3), hydrogen sulfide (H_2S), and greenhouse gas (GHG) emissions from concentrated animal feeding operations (CAFOs). This study was conducted to determine the effects of bedding material (corn stover (CS), bean stover (BS), wheat straw (WS), or pine wood chips (PC)) and ambient temperature (15°C (COOL) or 30°C (HOT)) on NH₃, CH₄, CO₂, N₂O, and H₂S flux in air samples collected in the headspace above lab-scale bedded packs. All bedded packs were housed at 18°C for an initial three weeks before being placed in their respective environmental chambers at 15°C or 30°C for the remainder of the 6-week study period. Significant two-way interactions of bedding material by temperature for NH_3 flux were observed (p = 0.0094). Ammonia flux was greater at higher temperature, while CS bedding had the lowest NH_3 emissions compared to the other bedding materials. A significant two-way interaction of bedding material by temperature for H_2S flux was observed (p < 0.0001), with significantly greater H_2S produced in the headspace of COOL-BS packs compared to all other treatments. Additionally, a significant (p = 0.0357) two-way interaction of temperature by age of the bedded pack was observed for H_2S flux. Hydrogen sulfide flux appeared to be influenced by low bedded pack pH to a greater extent than by increase in temperature. Greenhouse gas emissions tended to be higher from bedded packs in HOT treatments. A significant (p = 0.0422) interaction among bedding material, temperature, and age of the bedded pack was observed for CH₄. Significantly greater CH₄ flux was observed in the headspace above HOT-BS and HOT-CS at week 6 compared to all other treatments. A significant two-way interaction of bedding material by temperature was observed for CO_2 flux (p = 0.0189). The largest CO_2 levels were observed above WS bedding material regardless of temperature. Nitrous oxide flux decreased over the 6-week study for all bedded packs, while WS and PC bedded packs produced the greatest N_2O flux. The results indicate that feedlot operators maintaining bedded pack facilities will have the greatest reduction in NH_3 emissions when using CS bedding, regardless of ambient temperature. To reduce CH_4 emissions, producers should avoid allowing BS and CS bedded packs that are maintained for longer than six weeks in HOT (30° C) temperatures; frequent cleaning during summer months is recommended. Based on the CO_2 equivalents of CH_4 and N_2O , producers should consider PC as an option to reduce GHG emissions.

Keywords. Ammonia, Bedding age, Bedding type, Beef, Carbon dioxide, Greenhouse gas, Hydrogen sulfide, Methane, Nitrous oxide, Temperature.

Beef feedlot producers in the Upper Great Plains and Midwest regions of the U.S. are building beef confinement feeding facilities to address the environmental and management issues associated with livestock production. Slatted-floor facilities require no bedding, but mono-slope and hoop facilities are typically

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bedded for animal comfort. A variety of bedding materials are used, with the most common being crop residues such as corn stover (CS), bean stover (BS), and wheat straw (WS) (Doran et al., 2010). Livestock manure is recognized as a source of ammonia (NH₃) and greenhouse gas (GHG) emissions from animal agriculture (Miller and Varel, 2001; Chadwick et al., 2011), and the bedding-manure mixture in confinement beef facilities is a potential source of gas emissions. To reduce NH₃ and GHG emissions from concentrated animal feed operations (CAFOs), feedlot operators evaluate many management decisions that impact gases, including feed, facility cleaning, animal type and age, and type of bedding material. Bedding material type has been reported to influence gaseous emissions (Misselbrook and Powell, 2005; Garlipp et al., 2011; Spiehs et al., 2012, 2014b, 2016; Ayadi et al., 2015).

The GHG contribution from ruminants has been heavily focused on methane (CH₄) production from enteric fermentation (Johnson and Johnson, 1995; Kebreab et al., 2008), although microbial decomposition of manure produced at CAFOs also results in NH₃, CH₄, carbon dioxide (CO₂), and nitrous oxide (N2O) production (Mackie et al., 1998). Undigested dietary nitrogen excreted in urine and feces contributes to NH₃ emissions (Archibeque et al., 2007). Ammonia volatilization increases with increasing temperature, reduction in dry matter content, pH, air movement, and other factors (Meisinger and Jokela, 2000). Potential negative respiratory health effects from NH3 volatilization are a concern for confinement workers (Mitloehner and Calvo, 2008) and animals. Ammonia exposure of 300 ppm is considered immediately dangerous to life or health (IDLH) for humans by the National Institute for Occupational Safety and Health (NIOSH; Ludwig et al., 1994). Ayadi et al. (2015) reported that NH₃, CH₄, CO₂, and N₂O concentrations in the headspace above lab-scale bedded packs were higher at 40°C compared to 10°C. Bedded packs using CS compared to BS resulted in greater NH₃ concentrations when ambient temperatures were 40°C. Previously, Spiehs et al. (2016) reported that bedded packs containing greater than 20% pine chips (PC) in a CS mixture reduced NH₃ by up to 14%. Regardless of the percentages of PC and CS in the bedding mixture, GHG (CH₄, CO₂, and N₂O) concentrations throughout the 6-week bedded pack study did not differ (p > 0.61).

In addition to contributing to odor, hydrogen sulfide (H₂S) emissions from livestock facilities are a safety concern because H₂S exposure can cause human and livestock fatalities (Hooser et al., 2000). Hydrogen sulfide exposure of 100 ppm is considered IDLH for humans by NIOSH (Ludwig et al., 1994). Hydrogen sulfide emissions are associated with liquid manure (Parker et al., 2010). Bedded packs lack the large volumes of liquid manure found in slatted-floor deep-pit facilities or manure lagoons and lack of agitation of the liquid that causes air bubbles containing H₂S to burst, thereby releasing H₂S emissions (Ni et al., 2009). For these reasons, H₂S emissions are relatively low in bedded pack facilities. To date, no H₂S emission data have been reported for beef mono-slope barns with deep-pits. In bedded pack environments, H₂S flux is produced as a result of sulfate-reducing obligate anaerobic bacteria (Zang et al., 2017). Obligate anaerobic bacterial degradation of sulfur-containing

proteins and organic matter (OM) excreted by livestock consuming diets high in corn distillers grains with solubles (DGS) or sulfur amino acids (cysteine and methionine) has been reported as the main contributor to H_2S emissions (Li et al., 2011; Mackie et al., 1998).

The objective of this study was to determine the effects of bedding material and environmental ambient temperature on flux of NH₃, CH₄, CO₂, N₂O, and H₂S from bedded packs.

MATERIALS AND METHODS Bedding Material

Corn stover, bean stover, wheat straw, and pine wood chips were used in this lab-scale bedded pack study. Corn stover, BS, and WS were sourced locally and processed in an Earthquake chipper/shredder (Ardisam, Inc., Cumberland, Wisc.) before being sifted through a 6.35 mm screen to remove fines as described by Spiehs et al. (2013). Kiln-dried pine wood chips were purchased commercially (Ozark Shavings Co., Licking, Mo.). Composition of the bedding materials was previously reported by Spiehs et al. (2013).

LAB-SCALE BEDDED PACKS

Lab-scale bedded packs were built using 0.43 m high and 0.38 m diameter plastic containers with no lids (fig. 1), as previously described by Spiehs (2018). To allow air movement, each container had six 10 mm holes spaced equally around its circumference, approximately 50 mm from the top of the container (Spiehs, 2018). During gas flux sample collection, $5 L \min^{-1}$ of positive pressure was supplied with only 1 L min⁻¹ collected for sampling.

Initially, 400 g of bedding treatment material was used for the base bedded pack. Three times per week, on Monday, Tuesday, and Thursday for the Tuesday sample collection group (environmental chambers A and B) and on Tuesday, Wednesday, and Friday for the Wednesday sample collection group (environmental chambers C and D), 300 g of cattle feces and 300 mL of cattle urine (adjusted to pH 7.4) were added to the bedded pack surfaces (fig. 2). Feces and urine were obtained and composited separately from feedlot cattle consuming a typical late finisher diet containing a maximum



Figure 1. Lab-scale bedded packs containing corn stover, bean stover, wheat straw, or pine wood chips and stored at cool (15°C) or hot (30°C) temperatures in environmental chambers.

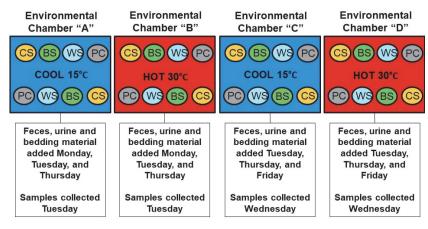


Figure 2. Lab-scale bedded pack layout, material additions, and sample collection times in environmental chambers A, B, C, and D at cool (15°C) or hot (30°C) temperatures. Bedding material was corn stover (CS), bean stover (BS), wheat straw (WS), or pine wood chips (PC).

of 30% wet corn distillers grains with solubles (WDGS). Bedding (200 g) was added once weekly on the day of sample collection after gas sample collection was completed. Following the addition of feces, urine, and bedding material, the bedded pack was mixed for 30 s using a plastic-covered 5.08 cm steel rod to imitate live animal hoof activity.

Air samples were collected from the bedded packs once weekly on day 28, 35, and 42 for NH₃, CH₄, CO₂, N₂O, and H₂S measurement before the addition of feces, urine, and bedding material. Pack temperature, pack height, and pH were also collected from the bedded packs once weekly on day 28, 35, and 42 before the addition of feces, urine, and bedding material. Each 6-week period used four environmental chambers, controlling ambient temperature and humidity (Brown-Brandl et al., 2011). Two replicates (environmental chambers B and D) were used for the hot treatment (HOT) at 30°C with a 12°C dew point, and two replicates (environmental chambers A and C) were used for the cool treatment (COOL) at 15°C with a 10°C dew point, with each chamber maintaining a steady ambient temperature and humidity level throughout the study. Each 1.6 m \times 1.6 m \times 2.44 m environmental chamber held eight lab-scale bedded packs, two of each bedding material treatment, during each of four 6-week periods, creating 16 experimental units per bedding treatment. During the first three weeks, before being placed in their respective environmental chambers, the bedded packs were all stored in a common room at constant temperature of 18°C.

GAS SAMPLE COLLECTION

Sample collections were completed on Tuesday (chambers A and B) and Wednesday (chambers C and D) before feces, urine, and fresh bedding were added to the bedded packs. Ammonia, CH₄, CO₂, N₂O, and H₂S flux were measured using eight stainless steel hemispherical flux chambers with an overall surface area of 640 cm² and 7 L volume (Spiehs, 2018). Air was collected from the headspace above each lab-scale bedded pack on days 28, 35, and 42. Flux samples from the air above the bedded packs were collected as described previously by Spiehs (2018) and Spiehs et al. (2019) for NH₃, H₂S, and GHG analyses.

At the initiation of gas sampling, one flux chamber was placed on top of each lab-scale bedded pack. Centered within the headspace, approximately 70 mm above the bedded pack surface, was a 40 mm, 12 V axial-flow fan moving approximately 130 L min⁻¹ of airflow from the surface to the top of the flux chamber (Spiehs et al., 2019). An elastic rubber skirt, 61 cm square with a 22.9 cm diameter hole in the center, was installed over the flux chamber to form a seal on top of the bedded pack container when the flux chamber was placed on the container for air sampling (Spiehs, 2018). Inert tubing (0.64 cm diameter) was attached to the flux chamber using inert compression fittings (Spiehs, 2018). The inert tubing was attached to a gas sampling manifold that fed into the air sampling equipment (Spiehs, 2018).

The gas sampling system was controlled by a 24 V programmable logic relay, which signaled multi-positional three-way solenoids to open and close one of eight air inlet lines on the gas sampling manifold (Spiehs, 2018). One line was opened at a time to allow air sampling from each bedded pack (Spiehs, 2018). Ambient air was flushed through the tubing at a rate of 5 L min⁻¹ for 30 min, followed by 1 L min⁻¹ air sampling for 20 min per bedded pack (Spiehs, 2018). Only sampling data from minutes 8 to 20 were analyzed to guarantee a representative sample from the tubing. The initial 7 min of sampling data were removed to allow steady state to be reached.

The equipment used for analyses of the air flux samples was as previously described by Spiehs (2018). Hydrogen sulfide air flux samples were analyzed using a Thermo Fisher 450i hydrogen sulfide/sulfur dioxide/combined sulfur pulsed fluorescence gas analyzer (Thermo Fisher, Waltham, Mass.). Ammonia emissions were determined using a Thermo Fisher 17i ammonia chemiluminescent gas analyzer, and CH₄ was measured with a Thermo Fisher 55i direct methane and non-methane hydrocarbon backflush gas chromatograph. The remaining GHGs (N₂O and CO₂) were measured using an Innova 1412 photoacoustic gas monitor (LumaSense Technologies, Santa Clara, Cal.).

BEDDED PACK SAMPLE COLLECTION

Chemical and physical properties of the bedded packs were measured weekly before the addition of bedding, urine, and fecal material. A representative sample of approximately 20 g was collected at the center of each bedded pack approximately 7.6 cm below the surface and diluted with distilled water at a 1:2 mass ratio, followed by pH determination using a pH/mV/temperature meter (IQ150, Spectrum Technologies, Inc., Plainsfield, Ill.). Pack temperature was also measured in the center of each pack at approximately 7.6 cm below the surface using the IQ150 pH/mV/temperature meter. Bedded pack height was measured prior to air sample collection to determine the pack depth. Mixing of the bedding, feces, and urine each week prevented a sampling hole from developing in the middle of the bedded packs.

The free air space percentage was determined through water displacement, which was measured on the final day of the study, as described by Spiehs et al. (2012, 2014a, 2014b). Air displacement volume provides a rough estimate of aerobic/anaerobic status within a bedded pack, with greater air displacement being more aerobic.

STATISTICAL ANALYSIS

8.6

Data were analyzed as a 2×4 factorial with repeated measures in time using the GLIMMIX procedure of SAS (SAS Institute, Cary, N.C.). The model included the effects of bedding material, temperature, age of bedded pack, replicate, period, bedding material × temperature, bedding material × age of bedded pack, and bedding material × temperature × age of bedded pack. Covariate structure was tested using the Toeplitz, autoregressive, and compound symmetry variance components to find the lowest Akaike information criterion (AIC) value. Compound symmetry was found to provide the lowest AIC value. Fisher's least significant difference test was used for means separation when significant differences were detected in the least squares means. The experimental unit was bedded pack. Individual treatments represent bedding material type, temperature, and age of bedded pack. Differences were considered significant when p < 0.05and tendencies at p-values ranging from p = 0.05 to p < 0.10.

RESULTS AND DISCUSSION Physical Properties

A significant (p = 0.0027) interaction for bedding material by temperature was observed for the pH of the bedded packs (fig. 3). Bean stover (BS) bedded packs maintained at COOL temperatures had significantly (p < 0.0001) lower pH compared to COOL-CS, COOL-PC, and COOL-WS (7.6, 7.9, 8.1, and 8.3 pH, respectively). Meanwhile, COOL-WS bedded pack pH was significantly (p = 0.0124) greater than the other COOL treatments. Bedded packs with the HOT-PC and HOT-WS treatments had similar pH, which was higher than the pH of the bedded packs with the HOT-PC had significantly (p < 0.0001 and p = 0.0014, respectively) greater pH compared to the COOL treatments with the same bedding materials, while HOT-CS and HOT-WS had pH values similar to the same bedding materials in the COOL treatments.

No significant multi-variable interactions were observed for bedded pack temperature (table 1). Wheat straw bedded packs had significantly (p < 0.0001) greater temperatures compared to the other three bedding materials, while BS maintained the lowest bedded pack temperature (23.1°C ± 0.9 °C). Bedded pack temperatures did not change significantly over time. Bedded packs in the COOL treatments had average temperatures of 18.8°C ± 0.2 °C. Similar bedded pack temperatures that were approximately 2°C to 3°C greater than the ambient temperature were reported previously when lab-scale bedded packs were housed at

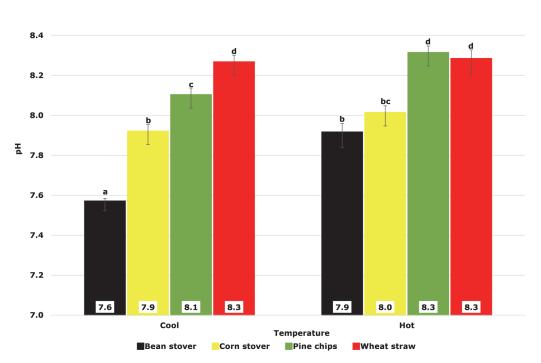


Figure 3. Average pH of lab-scale bedded packs stored at cool (15°C) or hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw over four 6-week periods. Values are least square means; error bars indicate standard errors. Within treatments, least square means with different letters are significantly different (p < 0.05). For bedding material × temperature interaction, p = 0.0027.

Table 1. Bedded pack pH, temperature, height, and free air space from lab-scale bedded packs containing bean stover, corn stover, pine wood chips, and wheat straw at 42 days.^[a]

		Bedded Pack	Bedded Pack	Bedded Pack	Free Air Space
Treatment Factors		pН	Temperature (°C)	Height (cm)	(%)
Bedding	Bean stover	7.7 ± 0.0	23.1 ±0.9 a	12.4 ±0.2 a	19.4 ±1.3
	Corn stover	8.0 ± 0.0	24.2 ±0.8 b	14.3 ±0.3 c	27.4 ± 1.1
	Pine wood chips	8.2 ± 0.0	23.7 ±0.8 ab	13.8 ±0.3 b	29.5 ±1.6
	Wheat straw	8.3 ±0.0	26.6 ±0.9 c	19.2 ±0.4 d	43.4 ± 0.8
Temperature	Cool (15°C)	8.0 ± 0.0	18.8 ±0.2 A	15.0 ±0.3	27.3 ±1.8
	Hot (30°C)	8.1 ±0.0	30.0 ±0.3 B	14.8 ±0.3	32.6 ± 1.6
Age of bedded pack	Week 4	8.0 ± 0.0	24.3 ±0.8	13.4 ±0.4 x	-
	Week 5	8.1 ±0.0	24.8 ± 0.8	14.9 ±0.4 y	-
	Week 6	7.9 ± 0.0	24.2 ±0.8	16.4 ±0.4 z	-
ANOVA Bedding Temperature		-	p < 0.0001	p < 0.0001	-
		-	p < 0.0001	NS	-
Age of bedded pack		-	NS	p < 0.0001	-
Bedding × Temperature		p = 0.0027	NS	NS	p = 0.0295
Bedding × Age of bedded pack		NS	NS	NS	-
Temperature × Age of bedded pack		NS	NS	NS	-
Bedding × Temperature × Age of bedded pack		NS	NS	NS	-

^[a] Treatments consisted of temperature (15° C or 30° C), bedding (bean stover, corn stover, pine wood chips, or wheat straw), and age of the bedded pack (4, 5, or 6 weeks old). Four 6-week experiments were conducted with four bedding material replicates per experiment (*n* = 128). Values are least square means with standard errors. Within a column, lowercase letters (a, b, c) indicate significant differences (p < 0.05) among bedding treatments, uppercase letters (A, B, C) indicate significant differences (p < 0.05) among age of bedded pack treatments. Significant effects (p < 0.05) are shown in bold.

approximately 18°C (Spiehs et al., 2013, 2014a, 2017). The slight increase in bedded pack temperature over ambient temperature is likely the result of heat generated by microbial activity. Conversely, bedded packs in the HOT treatments had average temperatures of 30.0° C $\pm 0.3^{\circ}$ C, which was identical to the ambient temperature.

When comparing bedding material types, the increases in bedded pack temperatures mirrored the increases in bedded pack temperatures mirrored the increases in bedded pack height (table 1). Bean stover bedded packs were significantly (p < 0.0001) shorter (12.4 ±0.2 cm) across bedding materials, while WS bedded packs were significantly (p < 0.0001) taller (19.2 ±0.4 cm) than the other three bedding materials. Bedded packs maintained at HOT and COOL temperatures were similar in height (14.8 ±0.3 vs. 15.0 ±0.3 cm). As anticipated, bedded pack height significantly (p < 0.0001) increased over time as bedding material was added throughout the 6-week study.

Free air space gives an indication of the bedded pack microbial environment, with lower free air space indicating greater potential for an anaerobic environment. The percentage of free air space was 2 to 4 times greater compared to previous free air space percentages from lab-scale bedded packs with similar bedding materials (Spiehs et al., 2013, 2014a). A significant (p = 0.0295) interaction for bedding material by temperature was observed for free air space as a percentage of total mass of the bedded pack (fig. 4). Free air space for COOL-WS and HOT-WS bedded packs was significantly (p < 0.0001) greater than for the other three bedding materials within their respective temperature treatments, while COOL-BS and HOT-BS bedded packs were significantly (p < 0.0001) lower in free air space compared to the other bedding materials within their respective temperature treatments. Across all treatments, HOT-WS had the greatest free air space at 44.9% significantly, while COOL-BS had the lowest at 16.0% significantly. Spiehs et al. (2013) reported that BS and WS had larger free air space in labscale bedded packs compared to CS and wood chips (15.8%,

14.0%, 8.8%, and 7.7%, respectively). Different bedding material batches between studies may have resulted in variable bedding material process lengths from one lab-scale bedded pack study to the next, contributing to some of the free air space differences observed.

AMMONIA

A significant (p = 0.0094) interaction between bedding material and temperature was observed for NH₃ flux (table 2). Ammonia flux from BS, CS, and WS was significantly greater at HOT temperatures than at COOL temperatures (fig. 5). Ammonia flux from PC was similar for HOT and COOL temperatures (303.7 and 281.4 mg m⁻² h⁻¹). The NH3 flux above COOL-CS was lowest (122.2 mg m⁻² h⁻¹) of all treatments, while HOT-WS had the greatest (372.9 mg m⁻ ² h⁻¹) NH₃ flux. Lower NH₃ flux from the COOL treatments was expected, as NH₃ volatilization increases as temperature increases (Ayadi et al., 2015; Koenig and McGinn, 2016). In a Nebraska open feedlot system, seasonal nitrogen loss during warmer summer months was 155 g head⁻¹ d⁻¹ compared to 92 g head⁻¹ d⁻¹ during colder winter months (Koelsch et al., 2018). Unused dietary nitrogen from cattle is primarily excreted as urea in urine (Varel, 1997; Bierman et al., 1999; Cole et al., 2005). As temperature increases, urease activity increases the rate of conversion of urea to NH₃ in manure (Moraes et al., 2017). Spiehs et al. (2011) reported similar findings from beef deep-bedded mono-slope facilities, where NH₃ concentrations in the air were 1694.6 \pm 49.4 ppm when the ambient temperature was at or above 20.6°C, compared to 252.1 \pm 92.0 ppm when the ambient temperature was at or below 0°C.

Ammonia emissions were potentially higher for the PC bedding treatments regardless of the temperature because of increased urine absorption, which allowed a greater percentage of the surface area of the urine-soaked bedding material to be exposed to air movement. When urine is not absorbed by the bedding material, it drains through to the bottom of

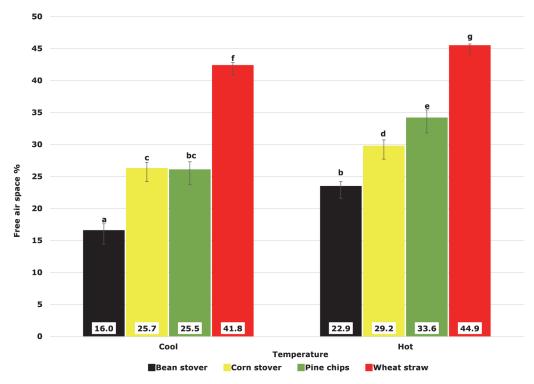


Figure 4. Free air space percentage of lab-scale bedded packs stored at cool (15° C) or hot (30° C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw at the end of the 6-week study. Values are least square means; error bars indicate standard errors. Least square means with different letters are significantly different (p < 0.05). For bedding material × temperature interaction, p = 0.0295.

Table 2. Interaction p-values for ammonia (NH₃), greenhouse gas (CH₄, CO₂, and N₂O), and hydrogen sulfide (H₂S) flux in the headspace air above lab-scale bedded packs containing bean stover, corn stover, pine wood chips, or wheat straw.^[a]

ab-scale bedded packs containing bean stover, corn sto	b-scale bedded packs containing bean stover, corn stover, pine wood emps, or wheat straw.									
Treatment Factors	NH_3	H_2S	CH_4	CO_2	N ₂ 0					
Bedding	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001					
Temperature	< 0.0001	<0.0001	<0.0001	< 0.0001	< 0.0001					
Age of bedded pack	0.0063	0.2310	<0.0001	0.1872	< 0.0001					
Bedding × Temperature	0.0094	<0.0001	0.0005	0.0189	0.1175					
Bedding × Age of bedded pack	0.5461	0.7644	0.0020	0.4823	0.8328					
Temperature × Age of bedded pack	0.4695	0.0357	<0.0001	0.3922	0.1028					
Bedding × Temperature × Age of bedded pack	0.9404	0.6385	0.0422	0.8783	0.9465					

^[a] Treatments consisted of temperature (15°C or 30°C), bedding (bean stover, corn stover, pine wood chips, or wheat straw), and age of the bedded pack (4, 5, or 6 weeks). Four 6-week experiments were conducted with four bedding material replicates per experiment (n = 128). Significant effects (p < 0.05) are shown in bold.

the bedded pack, where volatilization is limited due to less air turbulence (Misselbrook and Powell, 2005). Corn stover consistently provided some of the lowest NH₃ emissions in both COOL and HOT treatments, suggesting that CS is a viable bedding option to help reduce NH₃ volatilization.

HYDROGEN SULFIDE

A significant (p < 0.0001) interaction between bedding material and temperature was observed for H₂S flux in the headspace above the bedded packs (table 2). Hydrogen sulfide flux from bedding materials at COOL temperatures was greater for all treatments compared to HOT temperatures. The greatest flux (90.3 μ g m⁻² h⁻¹) was produced by COOL-BS, while HOT-WS produced the lowest flux at 5.6 μ g m⁻² h⁻¹ (fig. 6). The H₂S flux from HOT-WS was not statistically different from HOT-PC, HOT-CS, HOT-BS, and COOL-WS (15.2, 15.9, 17.3, and 20.7 μ g m⁻² h⁻¹, respectively).

Additionally, a significant (p = 0.0357) interaction between temperature and age of the bedded pack was observed for H₂S flux in the headspace above the bedded packs (fig. 7). Bedded packs stored at HOT temperatures had significant reductions in H₂S flux as the bedded packs increased in age from week 4 to week 6 (25.8 vs. 4.2 μ g m⁻² h⁻¹, respectively). Bedded packs stored at COOL temperatures were similar across all weeks, with week 6 having the highest flux (50.2 μ g m⁻² h⁻¹). At week 4, H₂S flux was similar between COOL and HOT treatments (41.7 and 25.8 μ g m⁻² h⁻¹, respectively). At weeks 5 and 6, bedded packs in COOL treatments produced significantly (p = 0.0013) greater flux compared to HOT treatments.

Previously, Arogo et al. (1999) reported that H_2S emissions from liquid swine manure were reduced as air temperature increased from 15°C to 35°C. However, H_2S emissions are typically affected by temperature in the opposite manner. Cortus et al. (2013) observed a significant increase in H_2S concentrations from mono-slope cattle facilities as temperature increased. As temperature increases, microbial activity increases, which stimulates H_2S production (Cortus et al.,

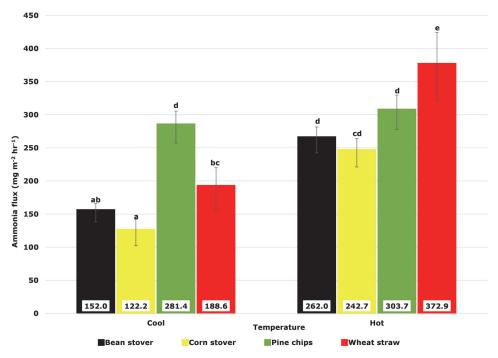


Figure 5. Average ammonia flux (mg m⁻² h⁻¹) in headspace air above lab-scale bedded packs stored at cool (15°C) or hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw. Values are least square means; error bars indicate standard errors. Least square means with different letters are significantly different (p < 0.05). For bedding material × temperature interaction, p = 0.0094.

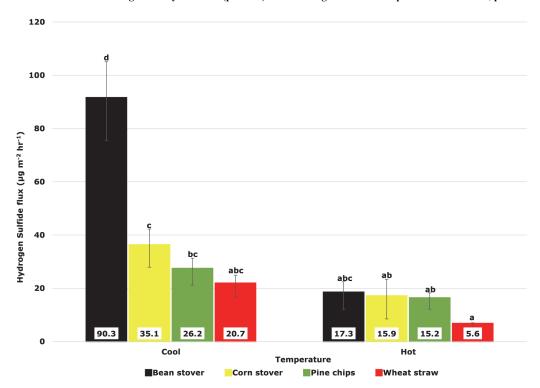


Figure 6. Average hydrogen sulfide flux (μ g m⁻² h⁻¹) in headspace air above lab-scale bedded packs stored at cool (15°C) or hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw. Values are least square means; error bars indicate standard errors. Least square means with different letters are significantly different (p < 0.05). For bedding material × temperature interaction, p < 0.0001.

2014). Additionally, Andriamanohiarisoamanana et al. (2015) reported an exponential increase in H₂S concentrations from liquid dairy manure as temperature increased from 8°C to 28°C. In the current study, WS produced the lowest H₂S flux of all bedding materials across both HOT and COOL treatments (20.7 and 5.6 μ g m⁻² h⁻¹, respectively).

Bedded packs with WS had the highest pack height (19.2 \pm 0.4 cm), which indicates less compaction, more free air space, and potentially a more aerobic environment that is therefore less conducive to anaerobic H₂S production. Conversely, BS bedded packs had the lowest heights (12.4 \pm 0.2cm) at the conclusion of the 6-week study (table 1),

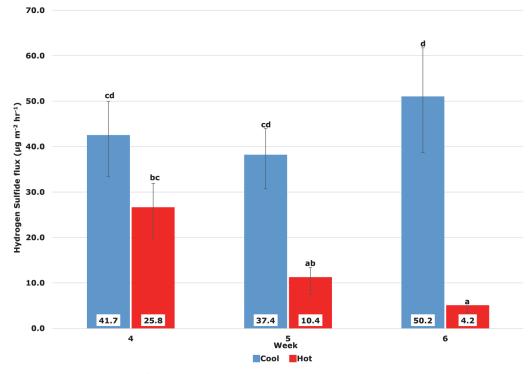


Figure 7. Average hydrogen sulfide flux (μ g m⁻² h⁻¹) in headspace air above lab-scale bedded packs stored at cool (15°C) or hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw. Values are least square means; error bars indicate standard errors. Least square means with different letters are significantly different (p < 0.05). For temperature × age of bedded pack interaction, p = 0.0357.

indicating increased compaction, less free air space, and a greater anaerobic microbial environment that contributed to the high H₂S flux from COOL-BS bedded packs.

In this study, low pH appears to have been the main influence on increased H₂S production, instead of the increase in temperature. The COOL-BS bedded packs had significantly lower pH (7.6 ± 0.0 pH) across all bedding materials and temperatures (fig. 4). Low pH (<7.0) favors H₂S production (Linderholm et al., 2008). Previous research demonstrated a 285% increase in H₂S concentrations when the pH of stored liquid dairy manure decreased from 7.32 to 6.82 (Andriamanohiarisoamanana et al., 2015). Yongsiri et al. (2004) reported that the effect of higher temperature on increased H₂S production was eliminated as the pH of wastewater sewage was reduced to 4.5. In the current study, the BS bedded packs were observed to have more standing liquid compared to the other bedded packs. Standing liquid provides more surface area for exposure to air movement, increasing H₂S volatilization and is a likely indication of anaerobic conditions in the bedding material.

GREENHOUSE GASES

A significant (p = 0.0422) interaction of bedding material, temperature, and age of bedded pack was observed for CH₄ flux in the bedded pack headspace (fig. 8). At week 4, CH₄ concentrations in the headspace above the bedded packs were similar across all treatments. At week 5, all the bedded packs in the COOL treatments and the packs in the HOT-BS, HOT-PC, and HOT-WS treatments produced similar yet minimal flux compared to HOT-CS. At week 6, HOT-BS and HOT-CS had significantly greater CH₄ flux (10.48 and 12.59 mg m⁻² h⁻¹, respectively) compared to all other treatments. HOT-BS and HOT-CS also had significantly greater CH₄ flux during week 6 compared to the previous weeks. Methane production is a result of anaerobic degradation of organic matter (Bryant, 1979). The BS and CS bedded packs had less free air space and lower heights at week 6 compared to the other treatments, which likely created an anaerobic environment conducive to CH₄ production (table 1). As expected, the bedded packs in the HOT treatments were significantly warmer than the bedded packs in the COOL treatments (30.0°C vs. 18.8°C, respectively) over the course of the study. The ideal temperature for mesophilic anaerobic CH₄ production from livestock manure is 35°C to 37°C (Sakar et al., 2009). Methane emissions from feedlot manure have been linked to increases in ambient temperature (Jarvis et al., 1995; Woodbury et al., 2018). Woodbury et al. (2018) reported that the mean CH₄ flux from feedlot manure increased exponentially from 0.004 to 0.572 g m⁻² d⁻¹ as the temperature increased from 5°C to 35°C.

A significant (p = 0.0189) interaction of bedding material and temperature was observed for CO₂ flux in the bedded pack headspace (fig. 9). Within the COOL and HOT treatments, CO₂ flux was significantly greater above WS (9164 and 9990 mg m⁻² h⁻¹, respectively) than the other bedding materials. Carbon dioxide emission is considered an indicator of microbial activity within livestock manure (Woodbury et al., 2018). The production of CO₂ is a result of aerobic respiration, which increases as temperature and aeration increase (Hao et al., 2001). When bedded packs are compacted or maintained at cold temperatures, aerobic respiration is reduced compared to more aerated or warmer bedded packs. In the current study, WS had greatest free air space in both HOT and COOL treatments (44.9% and

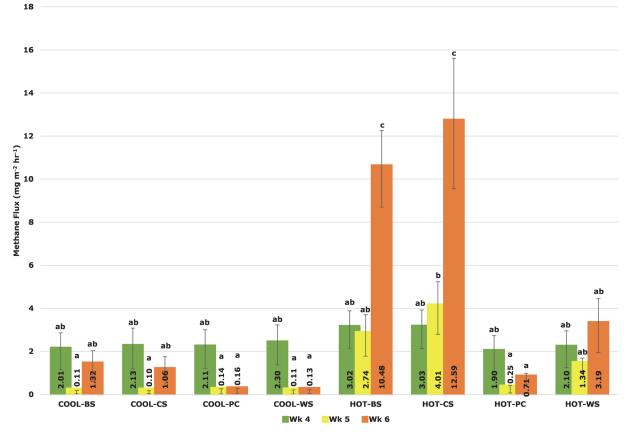


Figure 8. Average methane flux (mg m⁻² h⁻¹) over time in headspace air above lab-scale bedded packs stored at cool (15°C) or hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw. Values are least square means; error bars indicate standard errors. Within treatments, least square means with different letters are significantly different (p < 0.05). For temperature × bedding material × age of bedded pack interaction, p < 0.0422.

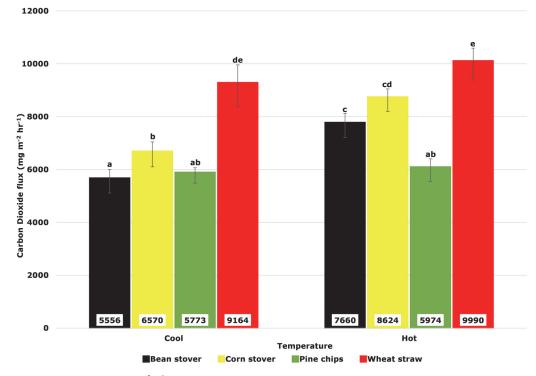


Figure 9. Average carbon dioxide flux (mg m⁻² h⁻¹) in headspace air above lab-scale bedded packs stored at cool (15°C) or hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw. Values are least square means; error bars indicate standard errors. Least square means with different letters are significantly different (p < 0.05). For bedding material × temperature interaction, p = 0.0189.

41.8%, respectively; fig. 7) compared to the other bedding materials. Greater bedded pack height for WS provided greater oxygen (O₂) availability within the bedded pack, improving the aerobic environment for CO₂ production. The production of CO₂ is more sensitive to O₂ availability than temperature. This could be the reason for the COOL-WS and HOT-WS treatments both having the greatest and similar CO₂ flux compared to all other treatments. This was additionally observed in CO₂ flux mirroring the free air space percentages (fig. 4). Additionally, COOL-PC and HOT-PC also had similar CO₂ flux (5773 vs. 5974 mg m⁻² h⁻¹, respectively). Meanwhile, HOT-BS and HOT-CS had significantly greater CO₂ flux compared to the same bedding materials at COOL temperatures. The BS and CS bedded packs had the lowest bedded pack heights, indicating greater compaction compared to WS and PC. With compaction limiting O2 availability for the BS and CS bedded packs, CO2 production was possibly driven by an increase in ambient temperature.

No significant multi-variable interactions were observed for N₂O flux in the bedded pack headspace (table 2). Previous research by Ayadi et al. (2015) using CS and BS as bedding materials at 10°C and 40°C did not observe a significant interaction between bedding material, ambient temperature, and age of the bedded pack for N₂O over a 6-week study. In the current study, N₂O concentrations ranged from 0.029 to 0.808 mg m⁻² h⁻¹ across all bedded packs, which is similar to the N₂O flux values reported by Spiehs et al. (2014b, 2016, 2019). Significant (p < 0.0001) differences in N₂O flux across bedding material types were observed (fig. 10). Bedded packs containing WS or PC has similar N₂O flux, which was significantly higher than the N₂O flux from bedded packs containing BS and CS (0.392, 0.366, 0.247, and 0.217 mg m⁻² h⁻¹, respectively). The sampling at week 4 produced greater (p < 0.0001) N₂O flux compared to weeks 5 and 6, and N_2O flux decreased as the packs aged (0.484, 0.246, and 0.186 mg m⁻² h⁻¹, respectively). Similarly, Parker et al. (2018) reported that N₂O levels decreased over 18 days as a result of denitrification within feedlot manure. Bedded packs in the HOT treatments produced significantly (p < 0.0001) greater flux compared to the COOL treatments $(0.449 \text{ vs.} 0.162 \text{ mg m}^{-2} \text{ h}^{-1}$, respectively). Nitrous oxide flux is produced by both nitrification and denitrification processes, which are influenced by moisture and temperature (Parker et al., 2017). Similarly, Woodbury et al. (2018) reported that N₂O flux from feedlot manure increased from 0.170 to 0.995 g m⁻² d⁻¹ as temperature increased from 5°C to 35°C. The change in ambient temperature at week 3, when the bedded packs were moved from 18°C to their respective treatment temperatures, may be the cause of the N₂O peaks at week 4. However, in a previous 6-week lab-scale bedded pack study, Spiehs et al. (2019) observed a spike in N₂O around week 3 followed by a decrease to week 6.

GLOBAL WARMING POTENTIAL

Global warming potential (GWP) provides the ability to compare gases based on how much energy the emissions of one ton of gas will absorb over given period of time (EPA, 2019). The GWP of N₂O is 298 times that of CO₂ for a 100year time scale (EPA, 2019). In the current study, the N₂O flux from the bedding materials containing BS, CS, PC, and WS resulted in CO₂ equivalents of 73.6, 64.7, 109.1, and 116.8 mg m⁻² h⁻¹, respectively, based on the GWP of N₂O. Meanwhile, the GWP of CH₄ is 25 times that of CO₂ for a 100-year time scale (EPA, 2019). The CH₄ flux from the bedding materials containing BS, CS, PC, and WS resulted in CO₂ equivalents of 81.9, 95.5, 22.0, and 38.3 mg m⁻² h⁻¹,

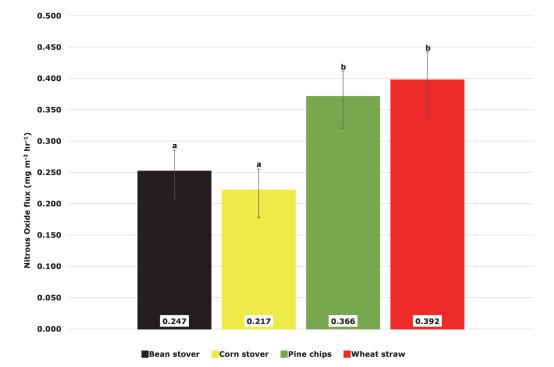


Figure 10. Average nitrous oxide flux (mg m⁻² h⁻¹) in headspace air above lab-scale bedded packs stored at cool (15°C) and hot (30°C) temperatures and containing bean stover, corn stover, pine wood chips, or wheat straw. Values are least square means; error bars indicate standard errors. Least square means with different letters are significantly different (p < 0.05). For bedding material difference, p < 0.0001.

respectively, based on the GWP of CH₄. Considering the total CO₂ equivalents of CH₄ and N₂O, the CS bedding material was the greatest at 160.2 mg m⁻² h⁻¹, while PC was the lowest at 131.0 mg m⁻² h⁻¹.

CONCLUSIONS

A significant (p < 0.0001) three-way interaction for bedding material by temperature by age of the bedded pack was observed for CH₄. Significantly greater CH₄ flux was observed in the headspace above HOT-BS and HOT-CS (10.48 and 12.59 mg m⁻² h⁻¹, respectively) at week 6 compared to all other treatments. A significant bedding material by temperature interaction was observed for NH₃, H₂S, and CO₂ (p = 0.0094, p < 0.0001, and p = 0.0189, respectively). Ammonia emissions were lower for the COOL treatments than for the HOT treatments throughout the study. Corn stover bedding produced the lowest NH₃ flux over time at both COOL and HOT temperatures (122.2 and 242.7 mg m⁻² h⁻¹, respectively). In bedded pack operations where NH₃ and respiratory issues are a concern, CS bedding material should be considered to minimize NH₃ volatilization, with the downside of increasing GHG release. Hydrogen sulfide flux was lowest from the HOT treatments, while WS bedding material produced the lowest H₂S flux in both COOL and HOT treatments (20.7 and 5.6 mg m⁻² h⁻¹, respectively). Significantly (p < 0.0001) greater H₂S was produced in the headspace of COOL-BS compared to all other treatments. Greenhouse gas emissions tended to be higher from bedded packs at HOT temperatures. The largest CO₂ levels were observed above WS, regardless of temperature. Nitrous oxide flux decreased over time for all bedded packs, while WS and PC bedded packs produced the greatest N₂S flux (0.392 and 0.366 mg m⁻² h⁻¹, respectively). Corn stover bedding material resulted is the greatest combined CO₂ equivalents for CH₄ and N₂O.

The results of this study indicate that feedlot operators maintaining bedded pack facilities will have the greatest reduction in NH₃ emissions when using CS bedding, regardless of the ambient temperature. To reduce CH₄ emissions, producers should avoid allowing BS and CS bedded packs to be maintained for longer than six weeks in HOT (30° C) temperatures; frequent cleaning during summer months is recommended. Based on the combined CO₂ equivalents of CH₄ and N₂O, producers should consider PC as option to reduce GHG emissions.

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