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Heidi Waldrip

David Parker

Sierra Miller

Lisa M. Durso

Byeng R. Min

See next page for additional authors

Authors

Heidi Waldrip, David Parker, Sierra Miller, Lisa M. Durso, Byeng R. Min, Daniel N. Miller, Kenneth Casey, Bryan Woodbury, and Mindy J. Spiehs

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Microbial community structure from southern High Plains beef cattle feedyard manure and relationship with nitrous oxide emissions

Heidi Waldrip[1] | **David Parker**[2] | **Sierra Miller**[3] | **Lisa M. Durso**[4] | **Byeng R. Min**[5] | **Daniel N. Miller**[4] | **Kenneth Casey**[6] | **Bryan Woodbury**[7] | **Mindy J. Spiehs**[7]

[1]USDA-ARS, Conservation and Production Research Laboratory, Bushland, TX 79012, USA

[2]West Texas A&M Univ., Canyon, TX 79016, USA

[3]Univ. of Texas Medical Branch, Galveston, TX 77550, USA

[4]USDA-ARS, Agroecosystem Research Unit, Lincoln, NE 68583, USA

[5]Tuskegee Univ., Tuskegee, AL 36088, USA

[6]Texas A&M AgriLife Research and Extension, Amarillo, TX 79106, USA

[7]USDA-ARS, Meat Animal Research Center, Clay Center, NE 68933, USA

Correspondence
Heidi M. Waldrip, USDA-ARS Conservation and Production Research Laboratory, PO Drawer 10, Bushland, TX 79012, USA.
Email: heidi.waldrip@usda.gov

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Abstract

Modern molecular techniques enable characterization of the microbial biome in livestock manure, from which there is particular concern over emission of greenhouse gases. This study evaluated how sampling depth, time, temperature, and artificial rainfall affected microbial community structure in feedyard manure, and relationships between the manure biome and known parameters related to nitrous oxide (N_2O) emissions. In three large incubation chambers, maintained at different temperatures that received two applications of artificial rainfall, we evaluated manure microbiome composition and abundance of N_2O-producing enzymes (*nirK* and *nirS*) using quantitative polymerase chain reaction (qPCR). These data were used with previously published data from the same study on N_2O emissions and assessment of manure physicochemical properties, denitrification enzyme activity (DEA), and nitrification activity (NA). Microbiome composition was *Firmicutes* (50%), followed by 32% *Actinobacteria*, 11% *Proteobacteria*, 5% *Bacteroidetes*, 1% *Chloroflexi*, and small populations (<0.5%) of *Planctomycetes*, *Deinococcus-Thermus*, *Gemmatimonadetes*, *Verrucomicrobia*, *Tenericutes*, and other organisms. Average bacterial populations varied largely as a function of sampling depth and time. *Firmicutes* increases tended to coincide with high N_2O emissions. Overall, the largest change observed was increased *Proteobacteria* at 5–10 cm, where relative abundance increased from 10% (17.2 ˚C) to 24% (46.2 ˚C) over time and with increased temperature. *Firmicutes* and *Actinobacteri*a predominated the microbial community of manure, but favorable conditions may lead to increases in *Bacteroidetes*, *Proteobacteria*, and *Chloroflexi*, which could influence N cycling and N_2O emissions from feedyards. Copy numbers of *nirS* at the beginning of the experiment

Abbreviations: AOA, ammonium oxidizing archaea; AOB, ammonium oxidizing bacteria; DEA, denitrifier enzyme activity; DNRA, dissimilatory nitrate reduction to ammonium; NA, nitrification activity; OM, organic matter; qPCR, quantitative polymerase chain reaction.

were higher than *nirK*. Differences in concentrations of *nirK* and *nirS* indicated that denitrifying enzymes in feedyard manure, particularly *nirS*, were sensitive to environmental changes.

1 | INTRODUCTION

The manure in beef cattle feedyards is a significant source of nitrous oxide (N_2O) (Parker, Casey, et al., 2017; Redding et al., 2015; Waldrip et al., 2017, 2020; Woodbury et al., 2018), a potent greenhouse gas (GHG) implicated in climate change due to a global warming potential (GWP) of 265–298 carbon dioxide equivalents (CO_2e). An estimated 25% of total GHG (N_2O and methane [CH_4]) from beef production originates from animal pens and housing (Costa et al., 2014). Studies have investigated the effects of temperature, rainfall, and activities of enzymes involved in feedyard N_2O emissions (Ayadi et al., 2015; Waldrip et al., 2020; Woodbury et al., 2001, 2018), but little work has characterized the manure microbiome or presence of specific genes responsible for N_2O production. In 2011, livestock manure contributed an estimated 4.3–5.8 Tg N_2O-N yr^{-1} globally (Syakila & Kroeze, 2011), which has been gradually and steadily increasing. Denitrification and nitrification are the two primary modes of N_2O production in soil, manure, sediments, and other substrates (Ayadi et al., 2015; Redding et al., 2015; Waldrip et al., 2017, 2020). In a previous work, we proposed that the majority of feedyard N_2O was derived from the top 5 cm of a feedyard manure pack and that it was likely derived from denitrification after rainfall (Waldrip et al., 2020). In the current study, we take this a step further and examine changes in the manure microbial community structure and abundances of genes involved in denitrification and nitrification. This data presented in this paper is one of the first phyla-level microbiome analyses of beef cattle manure in the Texas High Plains, where open-lot feedyards containing 100,000 or more cattle are common. In addition, the dairy industry is thriving in the region, with many open-lot dairies containing 3,000 or more milking cows.

In previous studies on manure N_2O emissions, we identified that substrate availability for microbial metabolism was highly related to feedyard manure N_2O emissions, denitrifier enzyme activity (DEA) and nitrification activity (NA) (Waldrip et al., 2017, 2020). Waldrip et al. (2017) conducted 15 nonflow-through–nonsteady-state measurement campaigns on two commercial Texas feedyards and developed predictive empirical models, where temperature and manure nitrate/nitrite (NO_x) and H_2O contents were positively related to measured N_2O emissions. In contrast, negative relationships were identified between N_2O and manure organic matter (OM), ammonia/ammonium (NH_x), dissolved organic carbon (DOC), and dissolved nitrogen (N) contents, as well as ultraviolet-visible (UV-vis) parameters related to OM complexity/availability. Differences in C and N availability for microbial growth and energy could impact microbial community structure, gene abundances, and activities of enzymes involved in denitrification and nitrification in feedyards. This study aimed to provide some answers to these questions.

Feedyard N_2O emissions are highly variable over both space and time (Aguilar et al., 2014; Parker, Casey, et al., 2017; Redding et al., 2015; Waldrip et al., 2016). This variability has been linked to temperature (Parker, Casey, et al., 2017, 2018; Waldrip et al., 2016, 2017, 2020), H_2O content (Aguilar et al., 2014; Parker, Casey, et al., 2017; Parker, Waldrip, et al., 2017, 2018), and manure characteristics (Ayadi et al., 2015; Liao et al., 2018; Waldrip et al., 2017, 2020; Woodbury et al., 2001). Both air temperature and precipitation range widely in the Texas Panhandle portion of the southern High Plains, where this study was conducted (−102.1, 35.2). This region is classified as semi-arid, with monthly average temperatures ranging from −7 °C in winter to 33 °C in summer. In addition to large seasonal changes, the region experiences extremes in daily temperatures, where it is not abnormal for summer temperature to change from 10 °C in the mornings, to above 39 °C by mid-afternoon. Precipitation in the region is sparse (average of 54 cm yr^{-1}) and occurs primarily as short, but often intense, rainfall episodes in the spring. Thus, the accumulated manure in open-lot cattle pens is subjected to both wet/dry cycling and temperature extremes.

Nitrous oxide is produced by numerous mechanisms, including nitrification, denitrification, coupled nitrification-denitrification, dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonia oxidation (Anammox), and various forms of chemodenitrification (Butterbach-Bahl et al., 2013; Giles et al., 2012). However, attempts to discern the processes involved in feedyard N_2O production have been largely inconclusive. The complexity and heterogeneity of feedyard pen manure and changing weather in a system exposed to ambient conditions, makes it unlikely that one process would be responsible for all feedyard N_2O. Multiple processes could be working simultaneously or in tandem. This complicates development of effective mitigation strategies, which generally require process-level knowledge of N_2O production mechanisms. In commercial feedyards the OM-rich manure is a complex ecosystem containing microbes, extracellular enzymes, both older and newly excreted feces and urine, and varying H_2O content. Furthermore, as feedyard manure

accumulates during the cattle finishing period, a layer structure forms with varied properties (e.g., density, porosity, OM complexity, NH_x and NO_x availability, microbial community composition, moisture content, and temperature) at different depths (Cole et al., 2009; Rice et al., 2007). It is unclear if N_2O production mechanisms differ with manure depth.

The fate of manure N depends on the consortia of microbial species present, activities of enzymes involved in OM degradation and N mineralization, manure chemistry, aeration (i.e., O_2 content), H_2O content, and substrate availability for microbial energy and respiration. Similar to soil observations, specific N_2O production pathways in manure vary with conditions and may occur consecutively or simultaneously in sequestered micro-sites within the manure pack (Azam et al., 2002; Lai and Denton, 2018; Wu et al., 2017). Some of the known N_2O production processes and genes involved are presented in Figure 1.

In soils, denitrification and nitrification are the predominant pathways of N_2O production. Nitrification occurs under aerobic conditions and is the stepwise oxidation of NH_4^+ or organic N to NO_3^- (Figure 1) by autotrophic bacteria and archaea, and heterotrophic fungi and bacteria. Nitrification requires enzymes for oxidation of NH_3 and hydroxylamine, which are encoded by *amoABC* and *hao* genes. Denitrification is a facultative respiratory pathway that occurs under anaerobic/anoxic conditions (or in anaerobic microsites) by a wide microbiome (Butterbach-Bahl et al., 2013; Jones et al., 2008; Shoun & Tanimoto, 1991; Zumft, 1997). In the absence of O_2, oxidized N species (i.e., NO_x, NO, and N_2O) are reduced and coupled to electron transport phosphorylation. Nitrous oxide is a frequent end-product if conditions are not optimal for nitrous oxide reductase (*nos*), which is the final enzymatic step of N_2O reduction to N_2 (Firestone et al., 1980). Complete denitrification generally requires a consortium of microbes. Denitrification steps and the genes responsible for enzyme synthesis have been well defined (Butterbach-Bahl et al., 2013; Ye et al., 1994). These are nitrate reductase (*nar*; NO_3^- → NO_2^-), nitrite reductase (*nir*; NO_2^- → NO), nitric oxide

Core Ideas

- Relationships were established among the beef manure microbiome, nitrous oxide losses, and environmental conditions.
- The predominant manure microbiome was *Firmicutes* > *Actinobacteria* > *Proteobacteria* > *Bacteroidetes* > *Chloroflexi* > others.
- Average microbial populations varied with sampling depth and time. Increases in *Firmicutes* coincided with nitrous oxide emissions.
- Copy numbers of *nirK* and *nirS* differed with date and among chambers, indicating sensitivity of denitrifying enzymes to environmental changes.

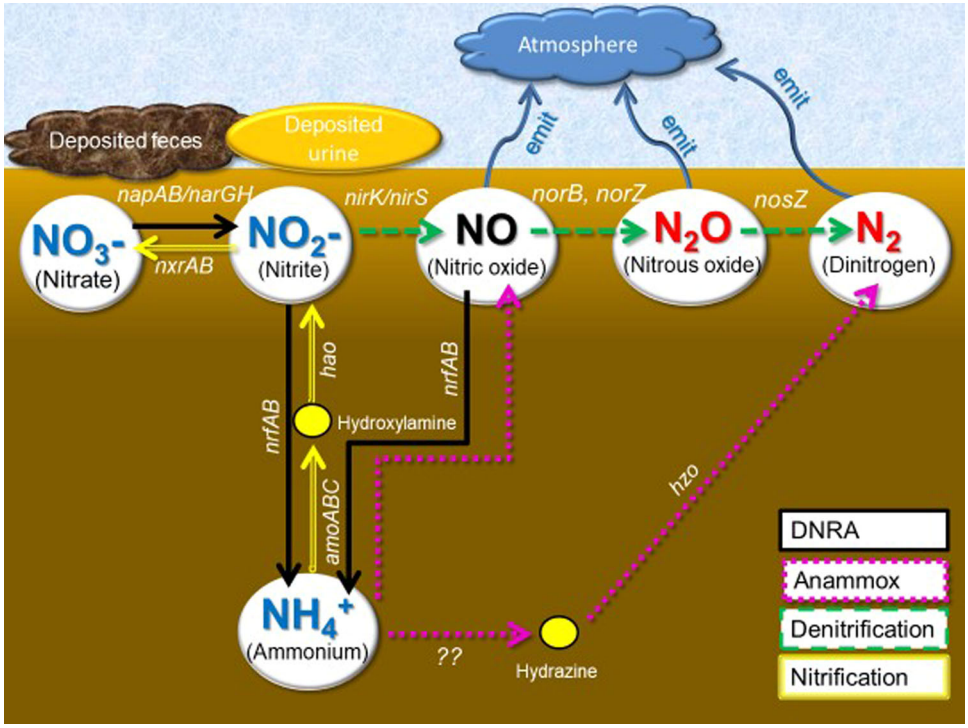

FIGURE 1 Diagram of major processes, compounds and genes implicated in manure nitrous oxide (N_2O) emissions

reductase (*nor*; NO → N_2O), and the previously mentioned nitrous oxide reductase (*nos*; N_2O → N_2) (Figure 1).

As N_2O production is largely biochemical, complex interactions exist among variables that affect microbial community structure and activity in manure. To date, no study has surveyed the microbiome of manure from open-lot beef cattle facilities and assessed the interaction among N_2O emissions, key variables, microbial populations, and enzyme activities. The objective of this study was to improve understanding of the composition of the microbial structure of beef manure and feedyard N cycling. We hypothesized that environmental conditions interact with manure properties dynamically, creating diverse microsites with differing N_2O-producing capacity. Once these interactions are better understood, targeted mitigation methods can be developed and evaluated to reduce feedyard N_2O-N losses and improve manure fertilizer N value.

2 | MATERIALS AND METHODS

2.1 | Large chamber incubation study

This work is a continuation of Parker et al. (2018) and Waldrip et al. (2020), who investigated N_2O emissions, manure physicochemical properties, and nitrification (NA) and denitrifier enzyme activities (DEA) in feedyard manure under different temperatures and following simulated rainfall. This report takes these works a step further to investigate how the manure microbiome responded and how specific microbial phyla and gene abundances are related to previously measured variables. For the basic chamber setup, manure that had accumulated during a typical 150-d finishing period was scraped from a pen at a commercial feedyard in the Texas Panhandle. The manure was a composite of unconsolidated, loose surface manure and deeper packed manure from cattle fed a steam-flaked corn (*Zea mays* L.)-based diet and housed at ~15 m^2 per animal. Selected physicochemical parameters of the manure were: 55% OM; 341 mg NH_x kg^{-1}; 5.7 mg NO_x kg^{-1}; 2.6% Total N; 27.7% Total C; and C/N of 10.8. Feedyard manure in this region is typically dry and hard packed. The manure was 91% dry matter (DM; as collected) and was roughly ground (<0.64 mm) and stored indoors to avoid further exposure to rainfall, temperature changes, and loss of gaseous N. Manure (109-mm depth) was added to each of three 1 m^2 (surface area) chambers. Compacted native caliche (calcium carbonate soil; 89 mm) underlaid the manure in each chamber to simulate the relatively impermeable soil-manure interface under open-lot feedyard pens (Mielke et al., 1974; Miller et al., 2008). The manure was compacted to an approximate dry bulk density of 0.61 g cm^{-3} with a handheld tamper. The experimental period was 59 d in length and occurred in winter and spring of 2017. Chamber temperatures were controlled with 1.3 by 1.3 by 1.3 m "hotbox" material warmers (Model HB64-1440, Powerblanket) with digital temperature controllers and 12 V exhaust fans for venting. In addition, silicone heating pads with digital temperature controllers (ProTherm Industries, Inc.) were attached under each chamber. A single chamber (Chamber 1) did not have thermal regulation and was exposed to ambient temperatures, which varied diurnally and seasonally in an indoor facility without temperature control.

To simulate the periodic wet/dry cycling that occurs in the region, we applied two episodes of simulated rainfall by evenly applying distilled H_2O, equivalent to a 25-mm rainfall event, to the surface of the manure with a handheld watering can on 13 February (Day 1) and 17 March (Day 22) of 2017. Manures dried naturally between H_2O applications. From Day 1 to 21 the three chambers were maintained at: (a) 5.0° (ambient), (b) 11.2°, and (c) 17.2 °C to simulate winter and mornings when temperatures are low. At Day 22, the second 25 mm of simulated rainfall was applied, and the temperatures of the chambers were increased and maintained at: (a) 15.0° (ambient, increased due to seasonal variation), (b) 38.1°, and (c) 46.2 °C until Day 59. This change was intended to simulate normal and extreme regional temperatures.

2.2 | Manure collection and analyses

Manure samples were collected in triplicate from chambers 2 h after H_2O application on Days 1 and 22, and then at 10:00 a.m. CST on Days 18 and 29. Manures were collected with a small trowel at depths of 0–5 cm and 5–10 cm by vertically inserting a 30 cm (height) by 7.6 cm (diameter) tin cylinder. Care was taken during sampling to avoid disturbance of the manure both inside (i.e., depth mixing) and near the cylinder (i.e., integrity of emitting surface). For each sample, manure from the top 5 cm was removed manually, placed in polyethylene bags, and then stored on dry ice. The 5-to-10-cm depth fraction was collected and stored in the same manner.

Basic manure properties were reported in detail in Waldrip et al. (2020). In brief, oxidation–reduction potential (Eh) was measured according to Brown et al. (2000) using a Pocket Pro ORP Tester (Hach Company). Manure pH was measured [1:10 (wt/wt) with deionized H_2O (pH 8.01)] with an Accumet XL250 pH meter and AccuCap combination pH electrode (Thermo Fisher Scientific). Manure DM was determined gravimetrically after drying overnight at 105 °C. Concentrations of OM were determined by Loss on Ignition at 500 °C. Total N and TC contents were measured with a varioMAX CN analyzer (Elementar Analysensyteme GmbH). The NH_x and NO_x were extracted from manure samples with 2.0 M potassium chloride (KCl) for 30 min and quantified colorimetrically with a SEAL Analytical AQ2 Discrete Analyzer (SEAL Analytical Inc.) and USEPA's Method 350.1 (NH_x)

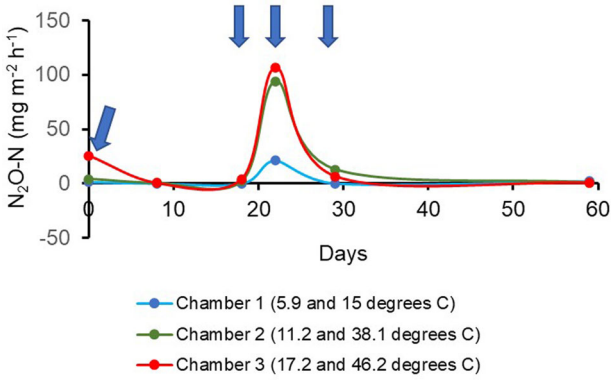

FIGURE 2 Nitrous oxide-nitrogen (N_2O-N) fluxes from feedyard manure following simulated rainfall application to chambers held at differing temperatures. Simulated rainfall episodes occurred on Days 0 and 22. Arrows represent manure sampling points. Modified from Parker et al. (2018)

and Method 353.1 (NO_x). Analyses of DEA and NA were conducted and reported in Waldrip et al. (2020) based on the methods of Woodbury et al. (2001) and Ayadi et al. (2015).

2.3 | Quantification of N cycling genes

Quantitative polymerase chain reaction (qPCR) was used to assess relationships between experimental parameters, targeted bacteria, and targeted bacterial functional genes. Standard qPCR was used to quantify *amoA* gene copy numbers for ammonium oxidizing archaea (AOA), ammonium oxidizing bacteria (AOB), and *nir*K and *nir*S (i.e., copper [Cu] and cytochrome cd1-type nitrite reductases, respectively). The qPCR assays were performed in triplicate, as described (Blaud et al., 2021).

3 | RIBOSOMAL RNA SEQUENCING FOR MICROBIAL COMMUNITY ANALYSIS

For 16S rRNA sequencing, triplicate sampled manures from each chamber/depth/date were composited into one sequencing run for each chamber/depth/date. These days largely coincided with measured peaks or lags in N_2O activity (Figure 2). The DNA was extracted from 200 mg bulk manure ($n = 25$) with a Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research) according to the manufacturer's protocol. Bead beating was done with a Precellys 24 (Bertin Instruments) for three 15 s cycles at 6,000 rpm. The DNA was eluted with 50 µl of DNA Elution Buffer and stored at −20 °C. Sequencing was conducted by MR DNA (Molecular Research LP). Bacterial 16S ribosomal RNA gene variable regions 1–3 were amplified using previously described primers (Dowd et al., 2008). An Ion Torrent PGM system (ThermoFisher Scientific) was used for sequencing according to manufacturer guidelines. Low quality reads were filtered from the dataset. Data were initially screened using Phred quality scores, which measure the identification quality of the nucleobases generated during sequencing. The Q25 sequences (indicating >99% base call accuracy) were retained for further processing using a proprietary analysis pipeline (www.mrdnalab.com). Following Q25 screening, data were further processed by removing barcodes, primer sequences, and sequences with fewer than 150 base pairs. Also removed were sequences with ambiguous base calls, homopolymer runs greater than 6 bp, chimeras (single DNA sequences originating from multiple transcripts or parent sequences), and singleton sequences. Operational taxonomic unit (OTU) clustering was performed at the 97% sequence similarity level (Capone et al., 2011; Eren et al., 2011; Swanson et al., 2011). Taxonomic assignments for each OTU were assigned using a BLASTn search against a curated database assembled from GreenGenes, RDPII, and NCBI (www.ncbi.nlm.nih.gov; DeSantis et al., 2006, http://rdp.cme.msu.edu).

3.1 | Nitrous oxide emission measurements

Headspace N_2O concentrations were measured from each chamber at 30-min intervals on the days of H_2O addition, and then daily for the remainder of the study. Details on the chamber system are available in Parker, Casey, et al. (2017); Parker, Waldrip, et al. (2017); and Parker et al. (2018). To measure emissions, chambers were fitted with a vented, portable lid that was moved among the chambers. Headspace air was recirculated from the sealed chamber with polyethylene tubing to a real-time N_2O analyzer (Model N2O/CO-30-EP Enhanced Performance, Los Gatos Research, Inc.). Concentrations of N_2O were recorded at 1-s intervals during 60-s measurement periods, with flux rates calculated from the slopes of N_2O concentrations vs. time using linear regression for 30-s periods. Emissions data were reported by Parker et al. (2018) and are presented in Figure 2. Chamber temperatures (± 0.1 °C) were monitored with thermistors (model no. ACC-SEN-SDIP, Acclima, Inc.) placed mid-depth in the manure of each chamber.

3.2 | Statistical analyses

As the collected data were not sufficient for full statistical analyses, we present survey information from two manure depths (0–5 cm, 5–10 cm) in three chambers that were subjected to six temperatures (5.9, 11.2, 15.0, 17.2, 38.1, and 46.2 °C) and two H_2O applications (Days 0 and 22). There were four manure sampling days (Days 0, 18, 22, 29). Data for gene copy numbers were log-transformed prior to

TABLE 1 Effect of sampling depth on bacterial phyla in feedyard manure. Population data were averaged across sampling times and chambers

	Proportion of total bacterial community				
Phyla	**0–5 cm**	**5–10 cm**	**Average**	**SEM**	***P* value**
Firmicutes	50.7	48.8	49.8	0.18	.18
Actinobacteria	32.3	31.9	32.1	0.49	.57
Proteobacteria	10.0	11.4	10.7	1.00	.32
Chloroflexi	1.10	1.20	1.10	0.07	.77
Bacteriodetes	4.60	5.30	4.90	0.24	.06
Planctomycetes	0.34	0.44	0.40	0.08	.38
Deinococcus thermus	0.10	0.11	0.10	0.02	.84
Gemmatimonadetes	0.09	0.11	0.10	0.02	.63
Verrucomicrobia	0.02	0.03	0.02	0.007	.17
Tenericutes	0.01	0.01	0.01	0.002	.92
Other	0.03	0.01	0.01	0.007	.05*
Firmicutes/Bacteriodetes ratio	11.0	9.20	–	–	–

Note. SEM, standard error of the mean.

*P value is statistically significant at $P < .05$.

analyses due to non-normality of the data. All data were analyzed using PROC GLM (SAS Institute). Pearson's correlation coefficients were employed to determine positive and negative relationships among variables with Proc CORR (SAS Institute). Qualitative inferences were made among measured variables and changes in prevalent microbial phyla.

4 | RESULTS AND DISCUSSION

4.1 | Microbial community composition

This study evaluated effects of sampling depth, time, temperature, and H_2O content on microbial community structure in feedyard manure and relationships with N_2O emissions. Bacteria were grouped into 10 main phyla (including unknowns) (Table 1, Figure 3). Average bacterial populations varied largely as a function of sampling depth and time (Table 2). There were no chamber × time interactions for the majority of the manure bacterial community, with the exception of *Bacteriodetes* ($P < .01$): this was more pronounced at 5–10 cm than at 0–5 cm. *Firmicutes* tended to increase at Day 22 at both 0–5 and 5–10 cm in all chambers, which coincided with high N_2O fluxes following the second H_2O addition and increased temperatures (Figure 2). At the end of the sampling period (Day 29), there were increased proportions of *Proteobacteria* and *Bacteroidetes* at 5–10 cm in all chambers, from ~7 to 20% (*Proteobacteria*) and ~5 to 8% (*Bacteroidetes*), with a decrease in *Firmicutes* from 52 to 39%. In addition, there were increased proportions of *Chloroflexi* and decreases in *Actinobacteria*. There were also small increases in the proportion of "All Other" microbes over time at both depths. Overall, the largest change observed was increased *Proteobacteria* at 5–10 cm: the relative abundance of *Proteobacteria* increased from 10.4% (17.2 ˚C) to 24.1% (46.2 ˚C) over time and with increased temperature in Chamber 3. These data suggested that *Firmicutes* and *Actinobacteria* predominate the microbial community of manure; however, favorable conditions may lead to growth and expansion of *Bacteroidetes*, *Proteobacteria*, and *Chloroflexi*, which could influence N cycling and N_2O emissions from feedyards.

The dominance of *Firmicutes* and *Bacteriodetes* in our collected samples was similar to that found in other ruminant manures (Bernhard & Field, 2000; Min, Solaiman, et al., 2014; Min, Wright, et al., 2014; Shanks et al., 2011). Both *Firmicutes* and *Bacteriodetes* have members that produce beneficial short-chain fatty acids (acetate, propionate, and butyrate) from relatively indigestible carbohydrates in the ruminant colon. *Bacteroidetes* isolated from agricultural soils, wastewater sludge, and feedyard manure express *nosZ* (Figure 1), although it is genetically divergent from that expressed by *Proteobacteria* (Jung et al., 2013). *Bacteriodetes* are of particular interest because the production of fatty acids in manure could provide a C source for nitrifiers and denitrifiers, leading to N_2O emissions. *Proteobacteria*, the third most common phyla observed in the manure at both depths, include pathogens as well as genera involved in N_2 fixation and transformation (e.g., *Nitrosomonas, Bradyrhizobium, Nitrospira, Nitrosolobus*, and *Nitrobacter*). *Actinomycetes* possess mycelia that grow in a web-like pattern similar to fungal hyphae, which increases direct contact with metabolic substrates. They are common in soil, sediments, and wastewater sludge and have denitrifiying capacity (Chèneby et al., 2000; Shoun & Tanimoto, 1991) and decompose complex OM, although they tend to exhibit slow growth and activity.

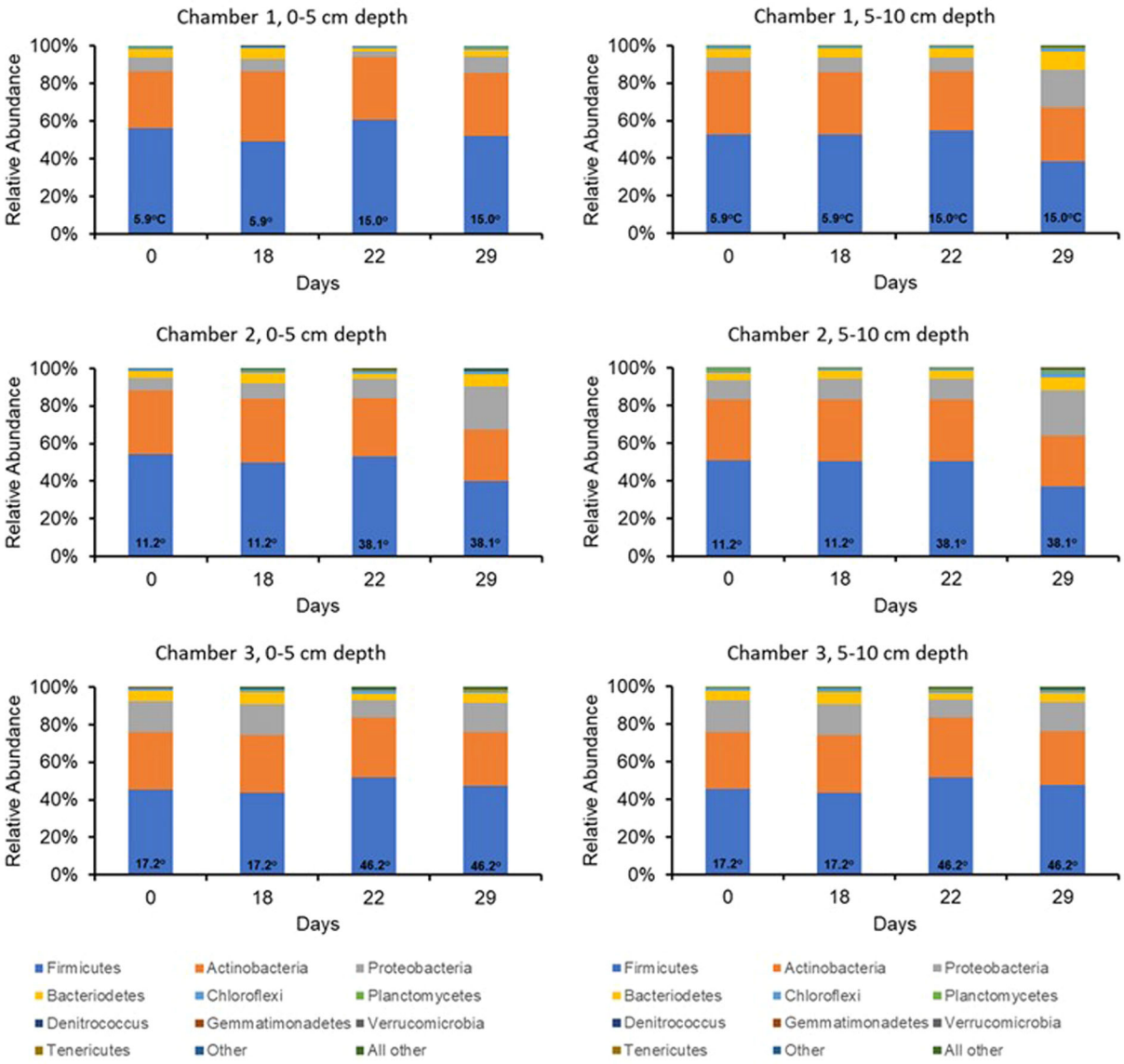

FIGURE 3 Relative abundance of major bacterial phyla in feedyard manure (0–5 and 5–10 cm depths) incubated for 29 d. Artificial rainfall events were applied of Days 0 and 22. In addition, temperature increases occurred in all chambers on Day 22

4.2 | Nitrous oxide gene quantitative polymerase chain reaction results

Biological denitrification involves a stepwise reduction of N oxides associated with electron transport phosphorylation and production of NO, N_2O, and N_2 in most situations. The reduction of NO_2^- to NO by nitrite reductase (*nirK* and *nirS*) is the first step that distinguishes denitrifiers from nitrate-respiring bacteria, which do not reduce NO_2^- to a gaseous form. Results from the current study indicate that *nirK* and *nirS* genes might influence competition with aerobic microorganisms and emission rates of N_2O. It has been reported that different denitrifying organisms (Abou-Seada & Ottow, 1988; Tiedje, 1988) and denitrifying communities in soils (Coyne et al., 1989) show differences in parameters that may influence competition with aerobic heterotrophs and N_2O emission rates (e.g., oxygen threshold, C requirement, and enzyme kinetics). Hence, knowledge of the underlying composition and diversity of denitrifier communities may help us better understand and manage N cycling in manure.

In this study, log-transformed average copy numbers of 16S rRNA, *amoA* from AOA (AOA-*amoA*) and AOB (AOB-*amoA*), and nitrite reductase genes (*nirK* and *nirS*) from Chambers 1, 2, and 3 at several key periods of N_2O emissions are presented in Table 3. At Day 0, there were 10.26 copies of 16S rDNA, with no significant change with incubation time ($P = .40$), chamber ($P = .62$), or time × chamber ($P = .34$). The log-transformed copy numbers of AOA-*amoA* ranged from 7.69 (Day 0) to 8.08 (Chamber 3, Day 18) and did not change significantly during the

TABLE 2 Effect of sampling depth and incubation time on bacterial phyla in feedyard manure. Population data were averaged across chambers. Days 0 and 22 were sampled 2 h after artificial rainfall

	Proportion of microbial community										
	Depth										
	0–5 cm					5–10 cm					
	Day										
Phyla	Initial	0	18	22	29	Initial	0	18	22	29	SEM
Firmicutes	49.5	54.8	47.3	55.2	46.7	49.5	52.6	50.8	51.2	40.4	2.05
Actinobacteria	32.9	32.2	33.9	32.7	29.9	32.9	33.3	32.4	32.9	27.8	1.04
Proteobacteria	9.7	6.8	10.2	7.4	15.6	9.7	7.6	9.6	9.6	20.4	2.13
Chloroflexi	1.1	1.1	1.2	1.1	1.1	1.1	1.0	1.1	1.1	1.6	0.16
Bacteriodetes	5.2	4.0	6.0	2.7	5.2	5.2	4.0	5.0	4.2	8.0	0.53
Planctomycetes	0.4	0.1	0.3	0.4	0.5	0.4	0.5	0.2	0.2	0.8	0.16
Deinococcus thermus	0.1	0.05	0.1	0.1	0.1	0.1	0.06	0.07	0.08	0.2	0.06
Gemmatimonadetes	0.1	0.04	0.07	0.1	0.1	0.1	0.07	0.06	0.06	0.2	0.05
Verrucomicrobia	0.02	0.05	0.01	0.01	0.04	0.02	0.02	0.01	0.01	0.09	0.001
Tenericutes	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.005
Other	0.01	0.04	0.02	0.01	0.08	0.01	0.01	0.01	0.01	0.02	0.01

incubation. Copy numbers of AOB-*amoA* were lower at 5.55 (Day 0), but similarly showed no significant change during incubation. However, average log-transformed AOB-*amoA* concentrations tended to increase significantly over time across all chambers ($P = .07$) and time periods ($P = .05$).

Copy numbers of *nirS* at the beginning of the experiment were higher (9.21) than *nirK* (7.89), indicating that the rate-limiting NO_2^- reduction step was dominated by bacteria, which produce both *nirK* and *nirS*, vs. fungi which only possess *nirK*. Soares et al. (2016) found no relationship between N_2O losses from a tropical soil and abundance of denitrification genes (*nirK*, *nirS*, *nosZ*); however, there was a positive correlation with *amoA* that suggested that nitrification by AOB was the main contributors to N_2O production. Differences in concentrations of *nirK* and *nirS* were slightly to highly significant ($P \leq .08$–$.01$) with time and chamber, indicating that denitrifying enzymes in feedyard manure, particularly *nirS*, were sensitive to environmental changes. Results indicated that *nirK* and *nirS* copy numbers might influence competition with aerobic microorganisms and N_2O emission rates. In soils, it has been reported that different denitrifiers and denitrifying communities have dissimilarities in oxygen threshold, C requirements, and kinetic parameters (Coyne et al., 1989; Tiedje, 1988). These preferential differences may influence competition with aerobic heterotrophs and N_2O emissions. Understanding the underlying composition and diversity of denitrifier communities may improve understanding and management of feedyard N cycling.

In a review on the soil N cycle, Hanke and Strous (2010) identified relevant bacterial phyla and processes involved in N cycling. The denitrifiers identified included *Alpha-*, *Beta-*, *Gamma-*, *Delta-*, and *Epsilon-Proteobacteria*, and *Nitrospira*. In addition, *Bacillus* can contain *nosZ* and have been classified as denitrifiers (Jones et al., 2008). Jones et al. (2008) examined pH effects on denitrification kinetics in soil *Bacillus* isolates and found that N_2O production tended to begin after 10 h of incubation with some, but not all, *Bacillus* strains if pH was 6.0, but N_2 was the predominant end-product at pH 7.0. These researchers speculated that an additional *nosZ* gene copy, expressed under different conditions, provided a competitive advantage and/or both genes were constitutively expressed under similar dentitrifying conditions.

The growth of NH_x oxidizers and denitrifiers are inhibited by temperatures >40 ˚C, particularly in bacterial and archaeal communities (Xu et al., 2017). In contrast, fungal communities, including *nirK*-producers, are more resistant to high temperatures and may proliferate at temperatures ranging from 10 to 40 ˚C in manure-amended soils. Thus, the N_2O-producing microbial community changes depending on environmental temperature. Lin et al. (2018) found that fertilization with swine manure slightly reduced AOA-*amoA* copy numbers compared with unfertilized control, but increased AOB. In addition, they found no relationship between AOA abundance and NA in swine manure-amended agricultural soils. In contrast, AOB, particularly *Nitrospira* Cluster 8a, had a positive relationship and was important in nitrification following fertilization with both organic and inorganic sources. Lin et al. (2018) also noted that AOB (primarily *Proteobacteria* including *Betaproteobacteria* and *Gammaproteobacteria*) abundance were strongly related to NO_3–N concentration and soil pH. Under aerobic conditions, biological NH_x oxidation is well documented in both natural and engineered

TABLE 3 Effect of sampling time and temperature on 16S rDNA and copy numbers of amoA from ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB), and nitrite reductase enzymes (*nirK* and *nirS*) in feedyard manure. Model effects (time, chamber, and time × chamber) were significant at $*P < .05$ and $**P < .01$)

Time	Chamber no. (temperature, ˚C)	16S rDNA	AOA-*amoA*	AOB-*amoA*	*nirK*	*nirS*
		Log copy no.[a]				
0	Initial	10.48	7.69	5.55	7.89	9.21
0 (2 h)[b]	1 (5.9)	10.10	7.83	5.39	7.95	9.21
	2 (11.2)	10.32	7.80	5.25	8.06	9.22
	3 (17.2)	10.27	7.84	5.47	7.79	9.11
18	1 (5.9)	10.25	7.75	5.20	7.71	8.99
	2 (11.2)	10.49	7.98	5.72	7.71	9.46
	3 (17.2)	10.45	8.08	5.19	8.14	9.52
22 (2 h)	1 (**15.0**)[c]	10.40	7.97	5.37	8.19	8.97
	2 (**38.1**)	10.29	7.96	5.79	7.74	9.34
	3 (**46.2**)	10.25	7.97	5.73	7.92	9.31
29	1 (**15.0**)	10.29	7.94	5.38	8.20	9.57
	2 (**38.1**)	10.30	7.98	5.90	8.30	9.76
	3 (**46.2**)	10.33	7.82	5.87	8.23	9.65
	SEM	0.018	0.023	0.191	0.122	0.294
ANOVA	Time	0.40	0.39	0.16	0.08	0.01**
	Chamber	0.62	0.67	0.13	0.07	0.05*
	Time × chamber	0.52	0.50	0.53	0.45	0.47
Averages						
Time	0	10.26	7.82	5.37	7.94	9.17
	18	10.39	7.93	5.36	8.01	9.32
	22	10.31	7.97	5.63	7.94	9.20
	29	10.30	7.91	5.72	8.24	9.66
	SEM	0.054	0.061	0.125	0.085	0.082
	P value	.10	.11	.07	.02*	.001**
Chamber	1	10.28	7.87	5.33	7.90	9.18
	2	10.34	7.93	5.67	8.17	9.44
	3	10.32	7.93	5.56	8.03	9.40
	SEM	0.047	0.053	0.109	0.074	0.071
	P value	.34	.45	.05*	.02*	.02*

Note. AOA, ammonia oxidizing archaea; AOB, ammonia oxidizing bacteria; *nirK*, copper nitrite reductase; *nirS*, cadmium nitrite reductase; 16S, 16S rRNA amplicon pyrosequencing; SEM, standard error of the mean.

[a]All values were log transformed prior to analysis. [b]Days 0 and 22 were sampled 2 h after artificial rainfall. [c]Changes in temperature are denoted in bold.

systems. Both AOA and AOB have been found to be involved in composting cattle manure (Yamamoto et al., 2010, 2012; Zeng et al., 2011); however, some studies (Posmanik et al., 2014; Yamada et al., 2013) identified that NH_x oxidation and nitrification in manure composts was related to AOB (*Nitrospira* and *Nitrosomonas*), but not AOA. Yamada et al. (2013) attributed the difference from other studies to high NH_x concentrations (>500 mg N kg^{-1}), as well as high NaCl and H_2O contents at the beginning of composting. This points to the importance of archaea in feedyard N_2O emissions, as AOA were identified as important NH_x oxidizers during the thermophilic phase (>50 ˚C) of composting (Jarvis et al., 2009; Yamamoto et al., 2012; Zeng et al., 2011) and in the digestion of poultry manure (Posmanik et al., 2014).

Although beyond the scope of the current study, another anaerobic N_2O-producing pathway is DNRA, which is carried out by obligate and facultative anaerobes, including *Clostridium*, *Desulfovibrio*, and *Enterobacteridae*, aerobes *(Bacillus, Pseudomonas, Arthrobacter, Nitrobacter)*, and *Ascomycota* fungi (Rütting et al., 2011; Tiedje, 1988; Zhou et al., 2002). In soils, DNRA is regulated by NH_4^+ and organic N concentrations and occurs under the same conditions as respiratory

denitrification (Rütting et al., 2011). Typically, N_2O from DNRA is distinguished from denitrification-derived N_2O by isotope tracer studies. Although DNRA has not been found to be a primary process for N_2O in soils, it is an important pathway in situations where the ratio of available C to electron acceptors (i.e., NO_3^-) is high, such as sediments, sludge digesters, and the bovine rumen (Kaspar & Tiedje, 1981; Shu et al., 2016). Thus, DNRA could be involved in N_2O production from anoxic zones deeper in the manure pack, where older OM has undergone more decomposition/fermentation to form available C and NO_3^-. Further study is required to explore the role of DNRA in feedyard emissions.

4.3 | Correlation coefficient (*R*) matrix

To further understand the effects of manure properties on N_2O emissions, microbiome changes, and nitrification and denitrification activities, measured parameter values were correlated against N_2O production (data not shown). Manure depth, DEA activity, and AOA and AOB copy numbers were not associated with N_2O production. However, manure temperature (R^2 = .63; P < .001), NH_x (R^2 = .66; P < .001), NO_x (R^2 = .73; P < .001), and nitrite reductase genes (*nirK* and *nirS*; R^2 = .57; P < .01) were positively related, and Eh (R^2 = −.39; P < .05), pH (R^2 = −0.34; P < .05), DM (R^2 = −.58; P < .01), and NA (R^2 = −.57; P < .01) were negatively related to N_2O production. *Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Deinococcus*, and *Gemmatimonadetes* had strong correlations with *nirK* and *nirS* gene copy numbers (R = .50–.79; P < .01–.001). The AOA-*amoA* and bacterial phylum (*Chloroflexi, Planctomycetes, Deinococcus*; R= .40–.68; P < .05–.01) and AOB-*amoA* and bacterial phylum (R = .41–.90; P < .05–.001; *Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Deinococcus*, and *Gemmatimonadetes*) were highly correlated. In addition, ratios of dissolved C/N in manure were negatively associated with NA (R = −.51; P < .05), *Firmicutes* (R = .49; P < .01), *Actinobacteria* (R = .40; P < .05), *Proteobacteria* (R = .41; P < .01), *Bacteroidetes* (R= −.46; P < .05), AOB-*amoA* (R = .47; P < .01), and nitrite reductase gene copy numbers (*nirK* and *nirS*; R = .44–.49; P < .05–.01). These results are consistent with other data (Waldrip et al., 2017), where an empirical model was developed to predict N_2O emissions based on temperature and manure NO_x and H_2O contents. In contrast, negative relationships were identified between N_2O and manure OM, NH_x, DOC, and dissolved N, as well as UV-vis parameters related to OM complexity/availability. Differences in C and N availability for microbial growth and energy could be involved in microbial community structure, gene abundances, and activities of enzymes related to N_2O emissions from feedyards. It is still unclear if N_2O production mechanisms differ with manure depth, but manure depth in the present study was negatively correlated (R^2 = −.75–.97; P < .001) with denitrification.

5 | CONCLUSIONS

This study evaluated how sampling depth, time, temperature, and H_2O content affected microbial community structure in feedyard manure, and relationships between the manure biome and known parameters related to N_2O emissions. The 16s ribosomal RNA gene sequencing was used to assess microbial community structure. The majority of the microbial community was comprised of *Firmicutes* (50%), followed by 32% *Actinobacteria*, 11% *Proteobacteria*, 5% *Bacteroidetes*, 1% *Chloroflexi*, and small populations (<0.5%) of *Planctomycetes*, *Deinococcus-Thermus*, *Gemmatimonadetes*, *Verrucomicrobia*, *Tenericutes*, and other organisms. Average bacterial populations varied largely as a function of sampling depth and time. There were no chamber × time interactions for the majority of the manure bacterial community, with the exception of *Bacteriodetes* (P < .01): this was more pronounced at 5–10 cm than at 0–5 cm. *Firmicutes* tended to increase at Day 22 at both 0–5 and 5–10 cm in all chambers, which coincided with high N_2O fluxes following the second H_2O addition and increased temperatures. Towards the end of the experiment, there were increased proportions of *Proteobacteria* and *Bacteroidetes* at 5–10 cm in all chambers, from ∼7 to 20% (*Proteobacteria*) and ∼5 to 8% (*Bacteroidetes*), with a decrease in *Firmicutes* from 52 to 39%. In addition, there were increased proportions of *Chloroflexi* and decreases in *Actinobacteria*. There were also small increases in the proportion of "All Other" microbes over time at both depths. Overall, the largest change observed was increased *Proteobacteria* at 5–10 cm: the relative abundance of *Proteobacteria* increased from 10.4% (17.2 ˚C) to 24.1% (46.2 ˚C) over time and with increased temperature in Chamber 3. These data suggested that *Firmicutes* and *Actinobacteri*a predominate the manure biome; however, favorable conditions may lead to growth and expansion of *Bacteroidetes*, *Proteobacteria*, and *Chloroflexi*, which could influence N cycling and N_2O emissions from feedyards and other open-lot cattle systems.

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AUTHOR CONTRIBUTIONS

Heidi Waldrip: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Supervision; Visualization; Writing-original draft; Writing-review & editing. David Parker: Conceptualization; Investigation; Methodology; Project administration; Supervision; Writing-review & editing. Sierra Miller: Data curation; Formal analysis; Investigation; Writing-review & editing. Lisa M. Durso: Data curation; Formal analysis; Investigation; Validation; Writing-review & editing. Daniel N. Miller: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Writing-review & editing. Byeng R Min: Formal analysis; Writing-original draft; Writing-review & editing. Kenneth Casey: Investigation; Methodology; Project administration; Validation; Writing-review & editing. Mindy J. Spiehs: Validation; Writing-review & editing. Bryan Woodbury: Conceptualization; Investigation; Methodology; Validation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Abou-Seada, M. N. I., & Ottow, J. C. G. (1988). Einfluß chemischer bodeneigenschaften auf ausmaß und zusammensetzung der denitrifikationsverluste drei verschiedener bakterien. (In German, with English abstract.) *Zeitschrift für Pflanzenernährung und Bodenkunde*, *151*, 109–115. https://doi.org/10.1002/jpln.19881510208

Aguilar, O. A., Maghirang, R., Rice, C. W., Trabue, S. L., & Erickson, L. E. (2014). Nitrous oxide fluxes from a commercial beef cattle feedlot in Kansas. *Air, Soil & Water Research*, *7*, 35–45. https://doi.org/10.4137/ASWR.S12841

Ayadi, F. Y., Spiehs, M. J., Cortus, E. L., Miller, D. N., & Djira, G. D. (2015). Physical, chemical, and biological properties of simulated beef cattle bedded manure packs. *Transactions of the ASABE*, *58*, 797–811. https://doi.org/10.13031/trans.58.10962

Azam, F., Muller, C., Weiske, A., Benckiser, G., & Ottow, J. C. G. (2002). Nitrification and denitrification as sources of atmospheric nitrous oxide - Role of oxidizable carbon and applied nitrogen. *Biology and Fertility of Soils*, *35*, 54–61. https://doi.org/10.1007/s00374-001-0441-5

Bernhard, A. E., & Field, K. G. (2000). Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Applied and Environmental Microbiology*, *66*, 1587–1594. https://doi.org/10.1128/aem.66.4.1587-1594.2000

Blaud, A., Maider, A., & Clark, I. M. (2021). Quantitation of ammonia oxidizing bacterial abundances in environmental samples by quantitative-PCR. *Methods in Molecular Biology*, *2232*, 135–146. https://doi.org/10.1007/978-1-0716-1040-4_12

Brown, H. A., Wagner-Riddle, C., & Thurtell, G. W. (2000). Nitrous oxide flux from solid dairy manure in storage as affected by water content and redox potential. *Journal of Environmental Quality*, *29*, 630–638. https://doi.org/10.2134/jeq2000.00472425002900020034x

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*, 20130122. https://doi.org/10.1098/rstb.2013.0122

Capone, K. A., Dowd, S. E., Stamatas, G. N., & Nikolovski, J. (2011). Diversity of the human skin microbiome early in life. *Journal of Investigative Dermatology*, *131*, 2026–2032. https://doi.org/10.1038/jid.2011.168

Chèneby, D., Philippot, L., Hartmann, A., Hénault, C., & Germon, J. (2000). 16S rDNA analysis for characterization of denitrifying bacteria isolated from three agricultural soils. *FEMS Microbiology Ecology*, *34*, 121–128. https://doi.org/10.1111/j.1574-6941.2000.tb00761.x

Cole, N. A., Mason, A. M., Todd, R. W., Rhoades, M., & Parker, D. B. (2009). Chemical composition of pen surface layers of beef cattle feedyards. *The Professional Animal Scientist*, *25*, 541–552. https://doi.org/10.15232/s1080-7446(15)30756-7

Costa, C. Jr., Li, C., Cerri, C. E. P., & Cerri, C. C. (2014). Measuring and modeling nitrous oxide and methane emissions from beef cattle feedlot manure management: First assessments under Brazilian condition. *Journal of Environmental Science and Health, Part B*, *49*, 696–711. https://doi.org/10.1080/03601234.2014.922856

Coyne, M. S., Arunakumari, A., Averill, B. A., & Tiedje, J. M. (1989). Immunological identification and distribution of dissimilatory heme cd1 and nonheme copper nitrite reductases in denitrifying bacteria. *Applied and Environmental Microbiology*, *55*, 2924–2931. https://doi.org/10.1128/aem.55.11.2924-2931.1989

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, *72*, 5069–5072. https://doi.org/10.1128/aem.03006-05

Dowd, S. E., Callaway, T. R., Wolcott, R. D., Sun, Y., McKeehan, T., Hagevoort, R. G., & Edrington, T. S. (2008). Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiology*, *8*, 125. https://doi.org/10.1186/1471-2180-8-125

Eren, A. M., Zozaya, M., Taylor, C. M., Dowd, S. E., Martin, D. H., & Ferris, M. J. (2011). Exploring the diversity of Gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. *PLOS ONE*, *6*(8), e26732. https://doi.org/10.1371/journal.pone.0026732

Firestone, M. K., Firestone, R. B., & Tiedje, J. M. (1980). Nitrous-oxide from soil denitrification -Factors controlling its biological production. *Science*, *208*, 749–751. https://doi.org/10.1126/science.208.4445.749

Giles, M., Morley, N., Baggs, E. M., & Daniell, T. J. (2012). Soil nitrate reducing processes drivers, mechanisms for spatial variation, and significance for nitrous oxide production. *Frontiers in Microbiology*, *3*, 407. https://doi.org/10.3389/fmicb.2012.00407

Hanke, A., & Strous, M. (2010). Climate, fertilization, and the nitrogen cycle. *Journal of Cosmology*, *8*, 1838–1845.

Iedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In A. J. B. Zehnder (Ed.), *Biology of anaerobic microorganisms* (pp. 179–244). John Wiley and Sons.

Jarvis, A., Sundberg, C., Milenkovski, S., Pell, M., Smars, S., Lindgren, P. E., & Hallin, S. (2009). Activity and composition of ammonia oxidizing bacterial communities and emission dynamics of NH_3 and N_2O in a compost reactor treating organic household waste. *Journal of Applied Microbiology*, *106*, 1502–1511. https://doi.org/10.1111/j.1365-2672.2008.04111.x

Jones, C. M., Stres, B., Rosenquist, M., & Hallin, S. (2008). Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Molecular Biology and Evolution*, *25*, 1955–1966. https://doi.org/10.1093/molbev/msn146

Jung, J., Choi, S., Jung, N., Scow, K. M., & Park, W. (2013). Primers for amplification of nitrous oxide reductase genes associated with Firmicutes and Bacteroidetes in organic-compound-rich soils. *Microbiology (Reading, England)*, *159*, 307–315. https://doi.org/10.1099/mic.0.060194-0

Kaspar, H. F., & Tiedje, J. M. (1981). Dissimilatory reduction of nitrate and nitrite in the bovine rumen: Nitrous oxide production and effect of acetylene. *Applied and Environmental Microbiology*, *41*, 705–709. https://doi.org/10.1128/aem.41.3.705-709.1981

Lai, T. V., & Denton, M. D. (2018). N_2O and N_2 emissions from denitrification respond differently to temperature and nitrogen supply. *Journal of Soils and Sediments*, *18*, 1548–1557. https://doi.org/10.1007/s11368-017-1863-5

Liao, W. H., Liu, C. J., & Gao, Z. L. (2018). Impacts of feedlot floor condition, deposition frequency, and inhibitors on N_2O and CH_4 emissions from feedlot dung and urine patches. *Journal of the Air & Waste Management Association*, *68*, 700–712. https://doi.org/10.1080/10962247.2018.1453392

Lin, Y. X., Ye, G. P., Luo, J. F., Di, H. J., Liu, D. Y., Fan, J. B., & Ding, W. X. (2018). Nitrosospira cluster 8a plays a predominant role in the nitrification process of a subtropical ultisol under long-term inorganic and organic fertilization. *Applied and Environmental Microbiology*, *84*, e01031–18. https://doi.org/10.1128/aem.01031-18

Mielke, L. N., Swanson, N. P., & McCalla, T. M. (1974). Soil profile conditions of cattle feedlots. *Journal of Environmental Quality*, *3*, 14–17. https://doi.org/10.2134/jeq1974.00472425000300010004x

Miller, J. J., Curtis, T., Larney, F. J., McAllister, T. A., & Olson, B. M. (2008). Physical and chemical properties of feedlot pen surfaces located on moderately coarse- and moderately fine-textured soils in southern Alberta. *Journal of Environmental Quality*, *37*, 1589–1598. https://doi.org/10.2134/jeq2007.0422

Min, B. R., Solaiman, S., Shange, R., & Eun, J.-S. (2014). Gastrointestinal bacterial and methanogenic archaea diversity dynamics associated with condensed tannin-containing pine bark diet in goats using 16S rDNA amplicon pyrosequencing. *International Journal of Microbiology*, *2014*, 141909. https://doi.org/10.1155/2014/141909

Min, B. R., Wright, C. K., Ho, P., Eun, J.-S., Gurung, N., & Shange, R. (2014). The effect of phytochemical tannins-containing diet on rumen fermentation characteristics and microbial diversity dynamics in goats using 16S rDNA amplicon pyrosequencing. *Agriculture, Food and Analytical Bacteriology*, *4*, 195–211.

Parker, D. B., Casey, K. D., Todd, R. W., Waldrip, H. M., Marek, G. W., Auvermann, B. W., Marek, T. H., Webb, K., Willis, W. M., Pemberton, B., & Meyer, B. (2017). Improved chamber systems for rapid, real-time nitrous oxide emissions from manure and soil. *Transactions of the ASABE*, *60*, 1235–1258. https://doi.org/10.13031/trans.12151

Parker, D. B., Waldrip, H. M., Casey, K. D., Todd, R. W., Willis, W. M., & Webb, K. (2017). Temporal nitrous oxide emissions from beef cattle feedlot manure after a simulated rainfall event. *Journal of Environmental Quality*, *46*, 733–740. https://doi.org/10.2134/jeq2017.02.0042

Parker, D. B., Waldrip, H. M., Casey, K. D., Woodbury, B. L., Spiehs, M. J., Webb, K., & Willis, W. M. (2018). How do temperature and rainfall affect nitrous oxide emissions from open-lot beef cattle feedyard pens? *Transactions of the ASABE*, *61*, 1049–1061. https://doi.org/10.13031/trans.12788

Posmanik, R., Gross, A., & Nejidat, A. (2014). Effect of high ammonia loads emitted from poultry-manure digestion on nitrification activity and nitrifier-community structure in a compost biofilter. *Ecological Engineering*, *62*, 140–147. https://doi.org/10.1016/j.ecoleng.2013.10.033

Redding, M. R., Devereux, J., Phillips, F., Lewis, R., Naylor, T., Kearton, T., Hill, C. J., & Weidemann, S. (2015). Field measurement of beef pen manure methane and nitrous oxide reveals a surprise for inventory calculations. *Journal of Environmental Quality*, *44*, 720–728. https://doi.org/10.2134/jeq2014.04.0159

Rice, W. C., Mason, A. M., Cole, N. A., & Clark, R. N. (2007). *The influence of feedlot pen surface layers on microbial community structure and diversity*. ASABE.

Rütting, T., Boeckx, P., Muller, C., & Klemedtsson, L. (2011). Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences*, *8*, 1779–1791. https://doi.org/10.5194/bg-8-1779-2011

Shanks, O. C., Kelty, C. A., Archibeque, S., Jenkins, M., Newton, R. J., McLellan, S. L., Huse, S. M., & Sogin, M. L. (2011). Community structures of fecal bacteria in cattle from different animal feeding operations. *Applied and Environmental Microbiology*, *77*, 2992–3001. https://doi.org/10.1128/aem.02988-10

Shoun, H., & Tanimoto, T. (1991). Denitrification by the fungus fusarium-oxysporum and involvement of cytochrome-p-450 in the respiratory nitrite reduction. *Journal of Biological Chemistry*, *266*, 11078–11082. https://doi.org/10.1016/S0021-9258(18)99130-1

Shu, D., He, Y., Yue, H., & Wang, Q. (2016). Metagenomic and quantitative insights into microbial communities and functional genes of nitrogen and iron cycling in twelve wastewater treatment systems. *Chemical Engineering Journal*, *290*, 21–30. https://doi.org/10.1016/j.cej.2016.01.024

Soares, J. R., Cassman, N. A., Kielak, A. M., Pijl, A., Carmo, J. B., Lourenco, K. S., Laanbroek, H. J., Cantarella, H., & Kuramae, E. E. (2016). Nitrous oxide emission related to ammonia-oxidizing bacteria and mitigation options from N fertilization in a tropical soil. *Scientific Reports*, *6*(11), 30349. https://doi.org/10.1038/srep30349

Swanson, K. S., Dowd, S. E., Suchodolski, J. S., Middelbos, I. S., Vester, B. M., Barry, K. A., Nelson, K. E., Torralba, M., Henrissat, B., Coutinho, P. M., Cann, I. K. O., White, B. A., & Fahey, G. C. (2011). Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *ISME Journal*, *5*, 639–649. https://doi.org/10.1038/ismej.2010.162

Syakila, S., & Kroeze, C. (2011). The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management*, *1*, 17–26. https://doi.org/10.3763/ghgmm.2010.0007

Tiedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In A. J. B. Zehnder (Ed.), *Biology of anaerobic microorganisms* (pp. 179–244). John Wiley and Sons.

Waldrip, H. M., Casey, K. D., Todd, R. W., & Parker, D. B. (2017). Nitrous oxide emissions from southern high plains beef cattle feedyards: Measurement and modeling. *Transactions of the ASABE*, *60*, 1209–1221. https://doi.org/10.13031/trans.12085

Waldrip, H. M., Parker, D. B., Miller, S., Miller, D. N., Casey, K. D., Todd, R. W., Min, B. R. J. S. M., & Woodbury, B. (2020). Nitrous oxide from beef cattle manure: Effects of temperature, water

addition and manure properties on denitrification and nitrification. *Atmosphere*, *11*, 1056. https://doi.org/10.3390/atmos11101056

Waldrip, H. M., Todd, R. W., Parker, D. B., Cole, N. A., Rotz, C. A., & Casey, K. D. (2016). Nitrous oxide emissions from open-lot cattle feedyards: A review. *Journal of Environmental Quality*, *45*, 1797–1811. https://doi.org/10.2134/jeq2016.04.0140

Woodbury, B. L., Gilley, J. E., Parker, D. B., & Stromer, B. S. (2018). Greenhouse gas emissions from beef feedlot surface materials as affected by diet, moisture, temperature, and time. *Transactions of the ASABE*, *61*, 571–582. https://doi.org/10.13031/trans.12483

Woodbury, B. L., Miller, D. N., Nienaber, J. A., & Eigenberg, R. A. (2001). Seasonal and spatial variations of denitrifying enzyme activity in feedlot soil. *Transactions of the ASAE*, *44*, 1635–1642. https://doi.org/10.13031/2013.7050

Wu, D., Cárdenas, L. M., Calvet, S., Brüggemann, N., Loick, N., Liu, S., & Bol, R. (2017). The effect of nitrification inhibitor on N_2O, NO and N_2 emissions under different soil moisture levels in a permanent grassland soil. *Soil Biology & Biochemistry*, *113*, 153–160. https://doi.org/10.1016/j.soilbio.2017.06.007

Xu, X., Liu, X., Li, Y., Ran, Y., Liu, Y., Zhang, Q., Li, Z., He, Y., Xu, J., & Di, H. (2017). High temperatures inhibited the growth of soil bacteria and archaea but not that of fungi and altered nitrous oxide production mechanisms from different nitrogen sources in an acidic soil. *Soil Biology & Biochemistry*, *107*, 168–179. https://doi.org/10.1016/j.soilbio.2017.01.003

Yamada, T., Araki, S., Ikeda-Ohtsubo, W., Okamura, K., Hiraishi, A., Ueda, H., Ueda, Y., Miyauchi, K., & Endo, G. (2013). Community structure and population dynamics of ammonia oxidizers in composting processes of ammonia-rich livestock waste. *Systematic and Applied Microbiology*, *36*, 359–367. https://doi.org/10.1016/j.syapm.2013.02.001

Yamamoto, N., Oishi, R., Suyama, Y., Tada, C., & Nakai, Y. (2012). Ammonia-oxidizing bacteria rather than ammonia-oxidizing archaea were widely distributed in animal manure composts from field-scale facilities. *Microbes and Environments*, *27*, 519–524. https://doi.org/10.1264/jsme2.ME12053

Yamamoto, N., Otawa, K., & Nakai, Y. (2010). Diversity and abundance of ammonia-oxidizing bacteria and ammonia-oxidizing archaea during cattle manure composting. *Microbial Ecology*, *60*, 807–815. https://doi.org/10.1007/s00248-010-9714-6

Ye, R. W., Averill, B. A., & Tiedje, J. M. (1994). Denitrification of nitrite and nitric oxide. *Applied and Environmental Microbiology*, *60*, 2560–2564.

Zeng, G. M., Zhang, J. C., Chen, Y. N., Yu, Z., Yu, M., Li, H., Liu, Z. F., Chen, M., Lu, L. H., & Hu, C. X. (2011). Relative contributions of archaea and bacteria to microbial ammonia oxidation differ under different conditions during agricultural waste composting. *Bioresource Technology*, *102*, 9026–9032. https://doi.org/10.1016/j.biortech.2011.07.076

Zhou, Z. M., Takaya, N., Nakamura, A., Yamaguchi, M., Takeo, K., & Shoun, H. (2002). Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. *Journal of Biological Chemistry*, *277*, 1892–1896. https://doi.org/10.1074/jbc.M109096200

Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews*, *61*, 533–616. https://doi.org/10.1128/.61.4.533-616.1997

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