

Experimental research on the effects of water application on greenhouse gas emissions from beef cattle feedlots

Orlando A. Aguilar · Ronaldo Maghirang ·
Steven L. Trabue · Larry E. Erickson

Received: 15 January 2014 / Accepted: 10 April 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract The effect of water application (e.g., through rainfall or sprinkler system) on emissions of greenhouse gases (GHGs), such as nitrous oxide (N₂O), methane (CH₄), and carbon dioxide (CO₂), from pen surfaces of open-lot beef cattle feedlots was evaluated under controlled laboratory conditions. Soil/manure samples were collected from several randomly selected pens from two beef cattle feedlots in Kansas and were used as simulated pen surfaces. Three treatments (i.e., dry and loose, moist and loose, and moist and compacted pen surface conditions) were considered, simulating surface conditions in the field after a typical rainfall event or water application with a sprinkler system. Soil/manure and water were mixed within glass containers and analyzed for GHG emission using a photo-acoustic infrared multi-gas analyzer; emission rates were calculated from measured concentrations. GHG emissions from the dry soil/

manure samples were low, with mean values of 0.02, 0.00, and 45 mg m⁻² h⁻¹ for N₂O, CH₄, and CO₂, respectively, compared to moist soil/manure samples. Water application on the dry manure samples resulted in large peaks of GHG fluxes, with peak values of 99.2, 28.6, and 15,443 mg m⁻² h⁻¹ for N₂O, CH₄, and CO₂, respectively.

Keywords Feedlot · Surface emission · Soil/manure drying process · Greenhouse gas emission · Rainfall effect

Abbreviations

<i>A</i>	Surface area (m ²)
AFOs	Animal feeding operations
<i>F</i>	Emission flux (mg m ⁻² h ⁻¹)
GHGs	Greenhouse gases
PIMA	Photo-acoustic infrared multi-gas analyzer
<i>S</i>	Slope of the least squares regression line between GHG concentration and time (ppm/min)
<i>V</i>	Volume of air within the static flux chamber (L)
ΔC	Gas concentration difference (ppm)
Δt	Sampling interval (min)

Introduction

Agricultural operations, including rice cultivation, soil management, and animal feeding operations (AFOs), account for a large part of the anthropogenic emissions of CH₄ and N₂O [1, 2]. AFOs, in particular, contribute to climate change and have become a public environmental concern [3] in many countries.

In most soil substrates, microorganisms play an important role in the production or consumption of N₂O, CH₄, and CO₂. The microbiological processes that are responsible for emissions of these GHGs (i.e., nitrification, denitrification,

O. A. Aguilar (✉)
Department of Mechanical Engineering, Universidad
Tecnológica de Panamá, Vía Ricardo J. Alfaro,
Campus Víctor Levi, Edif. #3, Panama, Republic of Panama
e-mail: orlando.aguilar@utp.ac.pa

R. Maghirang
Department of Biological and Agricultural Engineering,
Kansas State University, Manhattan, KS, USA
e-mail: rmaghir@k-state.edu

S. L. Trabue
USDA Agricultural Research Service, National Laboratory
of Agriculture and the Environment, Ames, IA, USA
e-mail: Steven.Trabue@ars.usda.gov

L. E. Erickson
Department of Chemical Engineering,
Kansas State University, Manhattan, KS, USA
e-mail: lerick@k-state.edu

methanogenesis, and respiration) are regulated by interactions among soil redox potential, pH, carbon (C) content, temperature, water content, and oxidants, including oxygen (O_2) and nitrate (NO_3^-) [4–6]. To survive, grow, and reproduce, most soil microorganisms need a source of C as a basic building block for new cells. These microorganisms obtain energy by catalyzing redox chemical reactions, in which inorganic compounds act as electron acceptors, allowing the complete oxidation of organic substrates, which act as electron donors [7]. To accomplish this process, electrons are transferred from the organic C substrate to an electron acceptor. Under aerobic conditions, most soil microbial cells use O_2 as an electron acceptor, releasing CO_2 into the atmosphere [6]. When the O_2 concentration within the soil decreases, as occurs in highly compacted or high water content substrates such as feedlot pen surfaces, the activity of aerobic microorganisms is depressed, but a special group of microorganisms capable of using NO_3^- as an electron acceptor can be activated. Further reductions of NO_3^- might result in a net emission of N_2O [6, 8]. If conditions within the soil become anaerobic for several days, methanogen cells will be activated to use hydrogen as an electron acceptor, resulting in CH_4 production [6].

Agricultural effects on GHGs emissions have been studied extensively [9, 10]. Peaks of N_2O emissions as much as 22 times larger than normal emission rates were obtained several days after rainfall in agricultural soils [11]. Other studies reported increased emissions of N_2O within minutes after adding water to dry agricultural soils [12, 13]. A study of GHG emissions from irrigated cropping systems as influenced by manure and synthetic fertilizer reported fluxes that were 55 times the mean values of the other plots [14]. This study also stated that the causes of those emission hotspots are generally unknown, and that those hotspots might be responsible for a very large proportion of the N_2O emissions. Increased microbial activity 8 h after watering dry soil has been reported [15]. De Klein et al. [16] also reported N_2O fluxes increasing from $20 \text{ g ha}^{-1} \text{ day}^{-1}$ before irrigation to $740 \text{ g ha}^{-1} \text{ day}^{-1}$, just 2 h after irrigation; they also reported that the flux increased up to $1,050 \text{ g ha}^{-1} \text{ day}^{-1}$, 24 h after the initial irrigation event.

Dusty conditions and heat stress are common challenges for cattle and feedlot operators during the summer season. Water sprinkling on pen surfaces is one of the best ways to reduce and control dust emissions [17, 18]. Sprinkling water on cattle also alleviates cattle heat stress [19]. Because GHGs are produced in the soil due to microorganism activity and because microorganism activity might be triggered by high water content, the potential for GHG emission while controlling dust or minimizing heat stress through water sprinkling must be evaluated. Despite extensive GHG emission research on soils, scientific information on GHG emissions from cattle feedlots,

particularly after a rainfall event or water application on pen surfaces, is limited. The main purpose of this study was to evaluate the effects of water application on GHG emissions from feedlot manure. This research is expected to contribute to a better understanding of the effects of water content and water application on GHG emissions from pen surfaces in beef cattle feedlots and related sources.

Materials and methods

Samples of beef cattle feedlot manure were collected from several pens in two beef cattle feedlots in Kansas. The samples were mixed and air-dried until the average gravimetric water content was approximately 0.10 g g^{-1} (wet basis). Large clods were removed by sieving using a 4.75-mm sieve. These processed samples were placed in glass containers and used as simulated pen surfaces, as described below.

Two sets of experiments were conducted (Table 1). The first set (Experiments 1a and 1b) involved determination of emission fluxes of N_2O , CH_4 , and CO_2 from the simulated dry pen surfaces after a rainfall event or water application. The second set (Experiments 2a and 2b) was designed to investigate the factors that influence the emission of those GHGs from the manure after water application.

Experiment 1: effects of water application on GHG emission fluxes

Experiment 1 had two parts (Table 1). The first part (Experiment 1a) assessed the long-term (up to 30-day) trend of emissions of N_2O , CH_4 , and CO_2 from simulated pen surfaces after water application. In this experiment, 218.8 g of the dry manure (0.10 g/g water content, wet basis) were placed in 1-L glass containers. There were three treatments, including the control, with three replications for each treatment. For the control (i.e., no water application), three containers with the dry manure were randomly selected. For the moist/loose manure treatment, three other containers were randomly selected and 111.2 g water at room temperature ($22 \text{ }^\circ\text{C}$) was added into the containers and slowly mixed with the dry manure. That amount of water represented a column of 16.7 mm of a simulated short-term but intense rainfall. Intense rainfall events between 8 and 22 mm were common in the field during the 2010 spring and summer seasons [20]. Final wet bulk density in the containers (Table 1) was within the range measured under field conditions, as described by Aguilar et al. [20]. For the moist/compacted manure treatment, samples were prepared in the same fashion as the moist/loose manure treatment, then immediately after mixing the water and the dry manure, samples were uniformly compacted until a wet bulk density of 1.1 g cm^{-3} was reached to simulate field

Table 1 Experimental parameters

Experiments	Treatments (pen surface conditions)	Manure conditions (wet basis)		Parameters measured		Sampling time
		Water content (g g ⁻¹)	Bulk density (g cm ⁻³)	Gases	Manure	
1a Effect of water application on greenhouse gas (GHG) emission fluxes	1-Control (dry/loose)	0.10	0.55	N ₂ O, CH ₄ , CO ₂	Temperature, water content	3.5, 6, 9, 24, 27, 48, 54, 72, 96, 120, 146, 172, 220, 314, 362, 410, 483, 531, 581, 720 h
	2-Moist/loose	0.40	0.69			
	3-Moist/compacted	0.40	1.1			
1b Effect of water application on GHG emission fluxes	1-Control (Dry/loose)	0.10	0.55	N ₂ O, CH ₄ , CO ₂	Temperature, water content	0.08, 0.25, 0.50, 0.75, 1, 1.5, 2, 3 h
	2-Moist/loose	0.40	0.69			
	3-Moist/compacted	0.40	1.1			
2a Mechanisms of GHG emissions from manure after water application	1-Control (dry/loose)	0.10	0.55	N ₂ O, CH ₄ , CO ₂	Temperature, water content, NO ₃ ⁻ , NH ₄ ⁺ , pH	0, 1, 4, 408, 720 h
	2-Moist/loose	0.40	0.69			
	3-Moist/compacted	0.40	1.1			
2b Mechanisms of GHG emissions from manure after water application	1-Control (dry/loose)	0.10	0.55	-	Temperature	Every 5 min for 45 days
	2-Moist/loose	0.40	0.69			
	3-Moist/compacted	0.40	1.1			

conditions. Compaction was performed manually using a cylindrical wooden stick and a rubber mallet. To standardize the compaction process, samples were compacted until a final volume of 300 cc of moist manure within the containers was reached. That final volume was computed based on manure physical conditions.

The first gas sampling and measurement for each container was conducted 3.5 h after water application. Immediately before sampling, each container was flushed with ambient air [21] to ensure that GHG concentrations at the headspace were at ambient levels. Sampling was performed using a photo-acoustic infrared multi-gas analyzer (PIMA; INNOVA 1312, AirTech Instruments, Ballerup, Denmark) equipped with optical filters for measuring N₂O, CH₄, and CO₂, and water vapor. Gas sampling was repeated within a period of 30 days, as shown in Table 1. Containers were kept uncapped within the laboratory during this period. Air temperature and pressure were measured during sampling. Soil/manure temperature from each container was also measured using a thermometer (model 14-983-17A, Fisherbrand, Pittsburgh, PA). Atmospheric pressure was measured using a barometer (Princo Southampton, PA).

The second part of the experiment (Experiment 1b) assessed the short-term (up to 3 h) effects of water application on GHG emissions. The experimental setup, including sample preparation, treatments, and instrumentation, was the same as that for Experiment 1a. Because of the higher sampling frequency in Experiment 1b, there were only two replications for each treatment. Gas sampling and

measurement was done at 0.08, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, and 3.0 h after water application (Table 1).

Experiment 2: mechanisms of GHG emissions after water application

Similar to Experiment 1, Experiment 2 had two parts. The first part (Experiment 2a) evaluated the mechanisms of GHG formation in the manure after water application. Treatments were the same as in Experiment 1. Twenty-four manure samples were prepared following the procedure described for Experiment 1. Five glass containers were used for the control (dry/loose manure and no water application).

GHG concentrations, manure physical and chemical characteristics (i.e., water content, temperature, pH, ammonium [NH₄⁺], and nitrate content [NO₃⁻]) were measured over the 30-day experimental period. Each container was sampled once, following the sampling scheme shown in Table 1. During sampling, the headspace gas concentration in the container was analyzed for GHG in the same manner as described for Experiment 1. After gas concentration measurement, a manure core was collected from the sampled container. Those cores were kept frozen, and at the end of the 30-d experimental period, they were analyzed at the Kansas State University Soil Testing Laboratory for pH, NH₄⁺, and NO₃⁻, as described by Aguilar et al. [20, 22]. Each container was discarded after core sampling. Manure temperature in each container was measured immediately before and after gas sampling using glass thermometers. The air temperature

and pressure in the laboratory were also measured using the same glass thermometers and the barometer, respectively, as described earlier.

Experiment 2b was conducted parallel to 2a. In contrast to Experiment 2a, in which manure temperature was measured only during gas sampling, manure temperature in Experiment 2b was measured continuously every 5 min for 45 days. Treatments were the same as described in Experiment 1, with two replicates each. Two different water applications were performed. The first water application was at 0 h; the second was at day 35 after the first water application. Manure temperature was measured using HOBO TMC6-HD sensors (-40 to 100 °C \pm 0.25 °C, resolution 0.03 °C) connected to a data logger (HOBO U12-008, Onset Computer Corporation, Bourne, MA).

Data analysis

The emission flux for each container during each sampling, F , was calculated from mass balance, as described by Aguilar et al. [22]:

$$F = k(V/A)(\Delta C/\Delta t) \quad (1)$$

where V is headspace volume, A is surface area, $\Delta C/\Delta t$ is the change in gas concentration with time within the container, and k is conversion constant. The slope (ppm min^{-1}) of the linear regression between gas concentration and time, S , was used for $(\Delta C/\Delta t)$ because the gas concentration was generally linearly related to time.

As described by Aguilar et al. [22], data were analyzed using Proc Glimmix of SAS with a 5 % level of significance and when the treatment \times time interaction was significant, and treatment differences were assessed for each sampling. Significant differences between treatments were determined using Tukey p value adjustments [23]. Correlation was assessed by Proc Corr of SAS [24]. The analysis of differences in the processes that generated time series soil/manure temperature was assessed by White Noise using R Project [25].

Results and discussion

Experiment 1: effects of water application on GHG emission fluxes

Figure 1 plots the emission fluxes for N_2O , CH_4 , and CO_2 as affected by water application. Emission fluxes from the dry/loose manure (control) were negligible. Application of water on the manure resulted in significantly larger emission fluxes for all three GHGs, suggesting that water application triggers GHG emission. Table 2 summarizes the mean and peak emission fluxes for Experiments 1a and 1b.

Nitrous oxide

Nitrous oxide emission flux for the control (dry/loose condition and no water application) was generally small (Fig. 1c, f). This result is consistent with the field measurements reported by Aguilar et al. [20] for cattle feedlots and by De Klein et al. [16] for soils. The N_2O emission fluxes from the moist/loose and moist/compacted manure samples were significantly larger than those for the control. Moreover, N_2O emissions from the moist/loose and moist/compacted manure samples did not differ significantly ($p > 0.05$); however, they differed significantly ($p < 0.05$) in the peak emission values. Figure 1c shows that for the dry soil/manure (control), the N_2O flux remained almost zero during the experimental period, but the N_2O fluxes from the moist/loose and moist/compacted manure increased to 99 and 74 $\text{mg m}^{-2}\text{h}^{-1}$, respectively, approximately 15 min after water application. The first N_2O peak from the moist/loose manure was significantly larger than that for the moist/compacted manure. The difference in the peak values between moist/compacted and moist/loose manure might be due to the larger wet bulk density of the moist/compacted manure (Table 1), which could have delayed gas diffusion from the substrates to their surface/air interface. Therefore, just the top layer of the moist/compacted manure was able to quickly diffuse N_2O to the headspace, which can also explain its quick and large N_2O flux decline during the first hour of the experiment. Previous researchers [11–13, 26–28] reported increased N_2O emission rates after rainfall events or artificial watering processes in agricultural soils. Nitrous oxide emission peaks as much as 22 times larger than normal fluxes were obtained at different times after a watering event [11]. Although several studies have reported large emissions of N_2O several hours or even several days after rainfall events, other studies, including Davidson [12] for dry grassland soil and Scholes et al. [13] for dry savanna soil, reported that emissions of N_2O began and markedly increased within minutes after adding water to soil at the end of the dry season. These results are comparable to those in the present study.

A second N_2O emission peak was observed for both the moist/loose and moist/compacted manure at 120 and 410 h after water application, respectively. The second N_2O peak for the moist/loose manure was observed when the N_2O flux of the moist/compacted manure and the control were not significantly different. The increased N_2O emission rate of the moist/compacted manure may be a consequence of the accumulated water underneath the surface due to soil compaction, which might have resulted in anaerobic conditions within the packed manure, triggering the denitrification process and enhancing N_2O emissions [11].

Fig. 1 Effects of water application on GHG emission fluxes: **a, b** and **c** correspond to CO₂, CH₄, and N₂O fluxes, respectively, during the first 3 h after water application (Experiment 1b); **d, e,** and **f** represent CO₂, CH₄, and N₂O fluxes, respectively, from 3.5 to 720 h after water application (Experiment 1a)

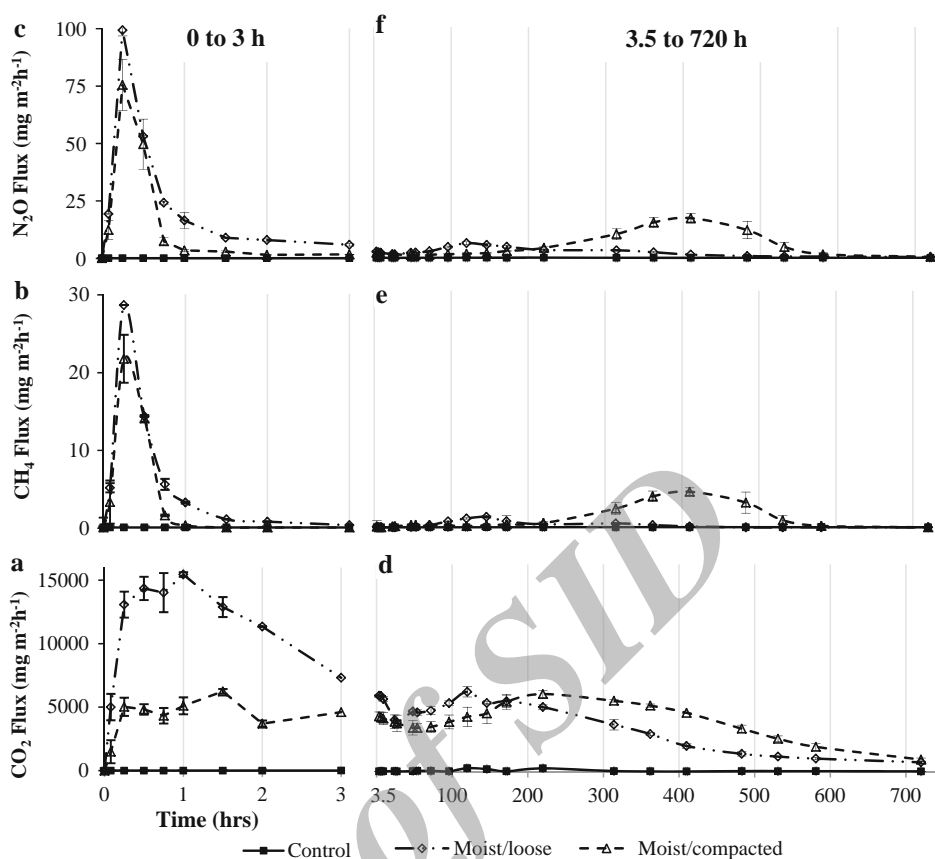


Table 2 Effects of water application on mean and peak emission values

Treatment	N ₂ O			CH ₄			CO ₂		
	Mean (mg m ⁻² h ⁻¹)	Peak (mg m ⁻² h ⁻¹)	Time [†] (h)	Mean (mg m ⁻² h ⁻¹)	Peak (mg m ⁻² h ⁻¹)	Time [†] (h)	Mean (mg m ⁻² h ⁻¹)	Peak (mg m ⁻² h ⁻¹)	Time [†] (h)
0 to 3 h after water application									
Dry/loose (control)	0.0	No peak		0.0	No peak		0.7	No peak	
Moist/loose	29.3	99.2	0.25	7.4	28.6	0.25	11,678	15,443	1.0
Moist/compacted	19.3	75.4	0.25	5.1	21.7	0.25	4,411	6,237	1.5
3.5 to 720 h after water application									
Dry/loose (control)	0.02	No peak		0.00	No peak		45	247	120
Moist/loose	2.60	6.38	120	0.29	1.33	146	3,935	6,153	120
Moist/compacted	4.33	17.2	410	0.89	4.51	410	3,894	5,980	220

[†] Time in which peaks were observed

After air-drying of manure, considerable NO₃⁻ as a result of nitrification is expected to remain within the manure; when water is applied under these conditions, denitrification might lead to a large N₂O production [11]. Therefore, the high N₂O emission rate within just 15 min after water application (Fig. 1c) likely was a consequence of a high concentration of NO₃⁻ in the dry manure, which suddenly triggered the activation of denitrification after the addition of water. Davidson [12] suggested that nitrifying and denitrifying microorganisms can survive for long periods of time in dry conditions and extreme high and low

temperatures and can become active within minutes after watering. In this experiment, as the moist/loose manure dried, conditions likely became more aerobic and reduced the denitrification activity, which could help explain the sustained reduction of N₂O emission flux 1 h after watering (Fig. 1c), reaching background levels 24 days later (Fig. 1f). In a soil normally dominated by air-filled pore space and oxidizing conditions, the soil may become saturated with water during recharge events, and reduced conditions and denitrification may dominate temporarily [29].

Methane

Figure 1b, e shows that CH₄ emission fluxes followed the same trend as N₂O fluxes. Emission flux from the control (dry/loose manure and no water application) was also negligible. Emission fluxes from the moist/loose and moist/compacted manure were significantly larger than that for the control. The first CH₄ emission peak from the moist/loose manure (28.6 mg m⁻² h⁻¹) was significantly larger than that for the moist/compacted manure (21.7 mg m⁻² h⁻¹), possibly as a consequence of the higher wet bulk density of the moist/compacted manure (Table 1). A second CH₄ emission peak was observed for both moist manure treatments at 120 and 410 h after watering, respectively (Fig. 1e). The second CH₄ emission peaks were smaller than the first. The CH₄ emission peak of the moist/compacted manure (4.5 mg m⁻² h⁻¹) was also significantly larger than that of the moist/loose manure (1.3 mg m⁻² h⁻¹). Results suggest that at 220 h after watering, the moist/compacted manure, which trapped water underneath the surface, could have become completely anoxic; the moist/loose manure had recovered its oxidizing conditions at that time. This is confirmed by the almost negligible CH₄ emission flux from the moist/loose manure, whereas the moist/compacted manure showed larger CH₄ emission flux at the same time, as shown in Fig. 1e.

As described by Li [6] and Saggari et al. [30], during a rainfall or watering event, the top surface layer might become saturated, and therefore the water would block the diffusion of O₂ into the soil profile, thus depleting the O₂ left in the soil pore space due to microbial consumption. Therefore, because microbial activity in the dry/loose manure is enhanced as soil water content increases, this might result in the formation of anaerobic microsites quickly following watering, which results in anoxic conditions in the soil [30]. Reduced conditions may dominate temporarily in a dry soil after watering [29]; furthermore, in the same manner as temporary anoxic conditions triggered denitrification, they also enhanced the activity of methanogenic bacteria, which resulted in large peaks of CH₄ fluxes in both moist treatments after watering.

Carbon dioxide

Carbon dioxide emission fluxes from all treatments and the control were significantly different (Fig. 1a, d). The larger CO₂ emission fluxes were observed from the moist/loose manure. Similar to N₂O and CH₄, CO₂ emission from the dry manure was negligible throughout the experimental period. Approximately 581 h after water application, emission fluxes from both moist treatments did not differ significantly from the control.

In the case of moist/loose manure, as soon as the pore space was filled with water, conditions could have become temporarily anoxic. Moreover, the water was exposed to vaporization because of the loose conditions and quickly moved deeper into the manure, which allowed O₂ diffusion from the air to the pore spaces and restored aerobic conditions in the substrate, as suggested by the wider CO₂ peak compared with the narrower N₂O and CH₄ peaks. Therefore, GHG emission fluxes from the moist/loose manure were likely the result of a combination of aerobic and anaerobic conditions present at the same time. Under aerobic conditions, most soil microbial cells use O₂ as an electron acceptor, thus releasing CO₂ into the atmosphere as its main respiratory product [6], so as expected, the largest CO₂ emission flux was observed for the moist/loose manure.

Carbon dioxide emissions from the moist/compacted manure were significantly smaller than those for the moist/loose manure during the first 3 h after water application (Fig. 1a). This might be due to the limited gas diffusion and anaerobic conditions. The manure's compacted condition, in addition to decreased gas diffusion (which limited O₂ diffusion), also retained anoxic conditions longer because of the trapped water. That sustained anoxic condition enhanced denitrification and methanogenesis, resulting in large emissions of N₂O and CH₄ but smaller emissions of CO₂. Figure 1d shows the decreasing trend of CO₂ emission flux for the moist/compacted manure 200 h after watering, whereas the emission fluxes of CH₄ (Fig. 1e) and N₂O (Fig. 1f) increased during the same time period. These results support the thesis of mostly anoxic conditions in the moist/compacted manure.

Experiment 2: mechanisms of GHG emissions after water application

Nitrous oxide

The control and moist treatments showed significant inverse correlation between manure NO₃⁻ and NH₄⁺ content (Fig. 2). Field measurements presented by Aguilar et al. [20] also indicated inverse, but non-significant, correlation between soil/manure NO₃⁻ and NH₄⁺. The non-significant inverse correlation between NO₃⁻ and NH₄⁺ from beef cattle pen surfaces was expected because of the likely constant soil/manure NH₄⁺ content with time as a result of the random and continuous inputs of fresh cattle urine and manure to the pen surfaces. No additional input of nitrogen came with time in this study; as such, a sustained decrease in soil/manure NH₄⁺ content was expected as nitrification increased with time.

The N₂O emission fluxes in Experiment 2 (Fig. 3f) followed the same trend as in Experiment 1. In Experiment

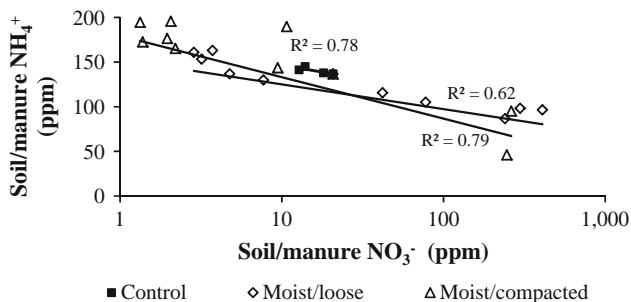


Fig. 2 Relationship between soil/manure ammonium and nitrate contents

2, the control (dry/loose manure) showed a significant, sustained small increase in NH_4^+ (Fig. 3d) and a significant, sustained, but small decrease in NO_3^- (Fig. 3e) during the 30-d experimental period. This result could explain the almost negligible emission of N_2O from the control in Experiments 1 and 2 (Figs. 1c, 3f) and suggests that even though conditions were aerobic in the control, nitrification was limited because nitrifying microorganisms were likely inactive as a consequence of the low water content. However, in both moist manure treatments, a sudden, although non-significant, decrease in NO_3^- occurred after watering (from 0 to 1 h); thereafter, a significant, large production of NO_3^- and a significant, large decrease in NH_4^+ were observed at the same time ($p < 0.05$) (Fig. 4e, d). These results suggest that although the manure was dry, both NO_3^- and NH_4^+ were being accumulated because only a small amount of denitrification occurred, but as soon as water was added, both nitrifying and denitrifying microorganisms were activated, as also suggested by the sudden increase of more than 2 °C within just 10 min of watering in both moist treatments (Fig. 3b). This may have triggered the transformation of NO_3^- into N_2O as byproduct of the denitrification process. Mikha et al. [15] reported increased microbial activity after watering dry soil, but 8 h after the watering event.

In the moist/loose manure, as suggested by the quick decrease in NO_3^- content after water application (Fig. 3e), sudden denitrification might be responsible for the large but narrow N_2O emission flux peak within the first 10 min after watering (Figs. 1c, 3f). That N_2O emission peak lasted for 30 min but quickly decreased to a minimum level, which was sustained during 120 h after watering. Up to 1 h after watering, the dominant process within the moist/loose manure was denitrification. One hour after watering, nitrification surpassed the rate of denitrification, as suggested by the significant decreasing rate of NH_4^+ content (Fig. 3d), whereas NO_3^- content increased significantly at the same time (Fig. 3e). At 120 h, when the manure water content began to decline steadily (Fig. 3a), aerobic

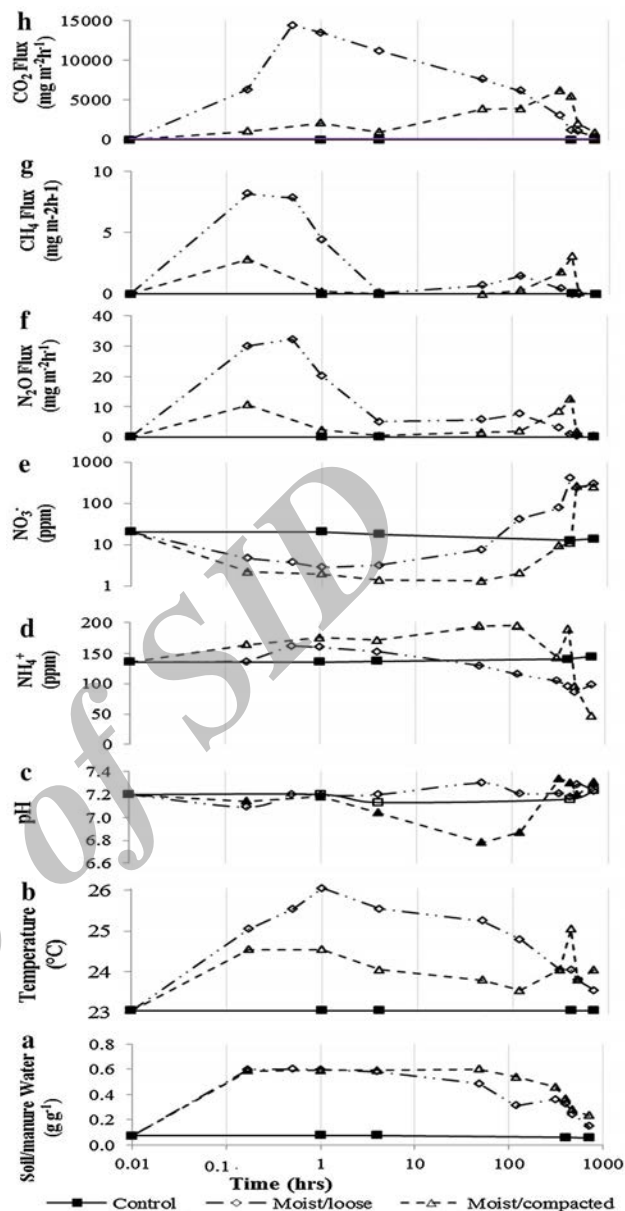
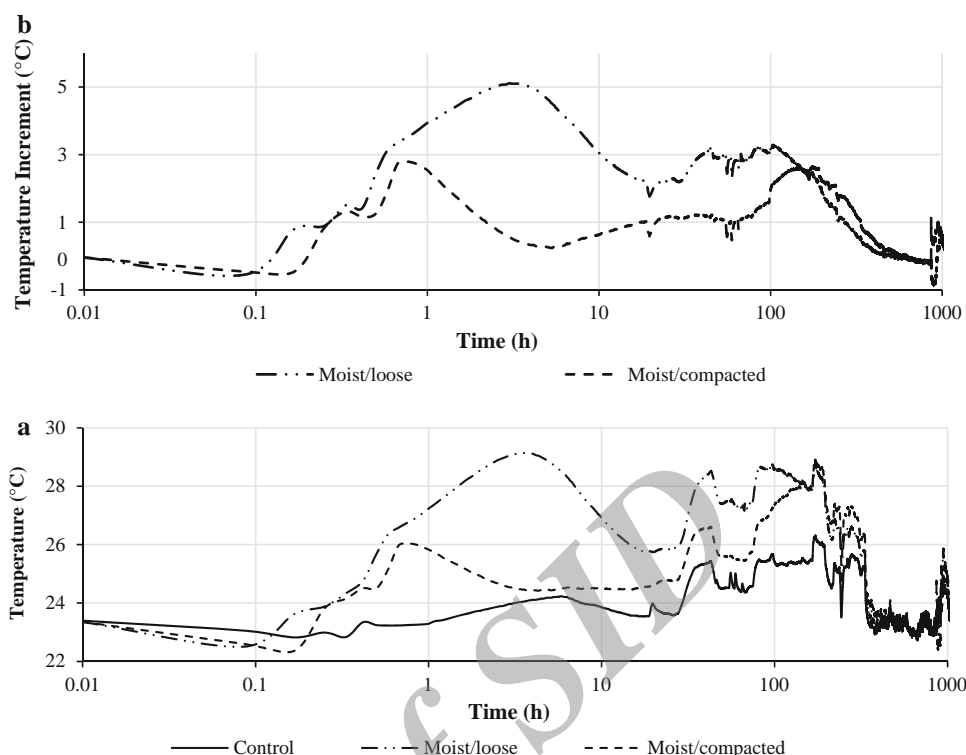


Fig. 3 Relationship among factors affecting N_2O emission flux versus time during 30 days after water application. **a** soil/manure, **b** soil/manure temperature, **c** soil/manure pH, **d** soil/manure NH_4^+ , **e** NO_3^- , **f** N_2O flux, **g** CH_4 flux, and **h** CO_2 flux

conditions dominated in the manure, then a sudden increase of NO_3^- content (from 42 to 409 ppm) was observed. In that same time period, N_2O emission flux decreased to a background level. A corresponding decrease in the manure temperature was also observed (Fig. 3b). These results suggest that 120 h after water application, aerobic conditions and, consequently, nitrification were predominant within the moist/loose manure and responsible for the decreased emission of N_2O .

As shown in Fig. 3c, the pH in the control was slightly alkaline during the experiment. Moreover, in both moist

Fig. 4 Soil/manure temperatures measured every 5 min during a 45-day period after two water applications: **a** total soil/manure temperature by treatment, **b** net temperature increment in the moist treatments with respect to the control



manure treatments, the pH decreased slightly with respect to the control as soon as water was mixed with the manure. In the moist/loose manure 1 h after watering, the pH increased above that of the control, reaching a maximum of 7.3 at 48 h after watering, then decreased to the background level. In the moist/compacted manure, the pH quickly decreased 1 h after watering, reaching a minimum of 6.8 at 48 h, then increased to 7.3 at 312 h after watering and remaining around that value until the end of the experiment. The lowest pH was observed for the moist/compacted manure. At the time of this minimum pH, the largest NH_4^+ content and the lowest NO_3^- content during the complete experimental period were also observed (Fig. 4c–e). In general, pH remained around 7, which is favorable for N_2O and CH_4 production [4].

The moist/compacted manure and the moist/loose manure behaved similarly, as shown in Fig. 3. Because rates of denitrification are higher with high water content [31] and anoxic conditions, during the first hour after watering, the denitrification process was stronger in this treatment than in the moist/loose manure, as suggested by Fig. 3e. Moreover, the narrow peak of N_2O emission flux was smaller (Fig. 3f), likely the result of reduced gas diffusion through the highly compacted surface. In this treatment, anaerobic conditions remained dominant until 408 h after watering. At 120 h, when the compacted manure started to dry, nitrification also took place, as suggested by the moist/compacted manure in Fig. 3e, with a sudden large increase of NO_3^- content. After 120 h, a

large N_2O emission flux began, with a large and broader peak at 408 h. That large N_2O emission peak might be the result of N_2O accumulation under the surface during the time that manure conditions were anoxic, which was released when the surface drying process began. The sustained (broader) peak also can be explained by the increase in manure temperature (Fig. 3b), suggesting that completely anoxic conditions were reached and maintained deeper in the manure after 120 h. Although the N_2O peak showed up at 408 h, nitrification was the dominant process in the manure surface with a large conversion of NH_4^+ into NO_3^- 120 h after watering, as suggested by Fig. 3d, f, whereas anoxic conditions persisted in the bottom section of the manure.

Several sources [11, 27, 28, 30, 32–36] have reported that N_2O is produced by the activation of both nitrification and denitrification processes. Groffman et al. [31], Kanako et al. [11], and Taghizadeh-Toosi et al. [37] reported that nitrification activity is activated under low water conditions and that it is enhanced by the presence of NH_4^+ , which results in the production of NO_3^- in the soil. They also suggested that denitrification is enhanced by the presence of a high amount of NO_3^- and that it is activated under high water content. Davidson [12] and Saggari et al. [30] reported that nitrification is dominant below field water capacity, whereas denitrification is dominant above field capacity. The formation of anaerobic sites following watering was responsible for N_2O emission rates up to 5 times larger when soil water content was above field capacity compared

with rates observed below field capacity [30]. This result suggests that well-drained pens in cattle feedlots will emit lower rates of N_2O than drained pens because the main driving agent in the dry pen is nitrification.

In general, as shown in Table 3, the N_2O emission flux from the moist/loose manure was directly correlated with manure factors such as water content, temperature, and NH_4^+ content and inversely correlated with pH and NO_3^- content. Ammonium was directly correlated with manure water content and temperature but inversely correlated with NO_3^- content. Nitrate content was inversely correlated with manure temperature. In the case of the moist/compacted manure, N_2O emission flux was significantly correlated only with manure temperature. Ammonium was significantly directly correlated with manure water content but inversely correlated with pH and NO_3^- content. Nitrate content showed a significant monotonic relationship with soil/manure water content (inverse) and pH content (direct), as indicated in Table 3; moreover, N_2O , CH_4 , and CO_2 emission fluxes were significantly directly correlated with each other.

Methane and carbon dioxide

The CH_4 and CO_2 emission fluxes in Experiment 2 (Fig. 3g, h) followed the same trends as those in Experiment 1, also displaying two different sets of gas emission peaks. Those sudden peaks of CH_4 and CO_2 emission fluxes after watering the dry manure also coincided with a sudden increase in manure temperature just 10 min after watering (Fig. 3b). As in Experiment 1, the CH_4 emission peak of both moist manure treatments reached the background level (control) 1 h later. Temperature in the moist/compacted manure also declined to the background level, suggesting little microorganism activity at that time.

In the moist/loose manure, after the first CH_4 and CO_2 emission peaks, the temperature steadily decreased and the CH_4 emission flux declined to the background level. The CO_2 emission flux, on the other hand, although decreasing, was still important 408 h later, when it also reached its background level. These results suggest that conditions in the manure became progressively more aerobic as the water content decreased. This trend also matched the large nitrification activity previously suggested in the same period of time.

In the moist/compacted manure, the temperature began to steadily increase 120 h after watering, reaching a maximum of 25 °C at 408 h, 2 °C above room temperature (Fig. 3b). This second increase in temperature might have resulted from increasing microorganism activity deeper in the manure after several days of high water content and limited gas diffusion through the manure. At that time, a second and broader CH_4 emission peak was reached. A CO_2 emission peak coinciding with the CH_4 emission

peak was also observed. This suggests that two different conditions were reached simultaneously in the vertical manure profile of the moist/compacted manure. At the surface, aerobic conditions increased as water evaporated; this substrate section might be responsible for the increasing CO_2 emission peak as well as for the nitrification activity previously reported for this treatment during the time interval. Furthermore, conditions became strongly anoxic deeper in the manure, a condition responsible for the increase in substrate temperature as well as for the CH_4 emission peak at that time interval.

As described by Paul [38] and Segers [39], microbial production of CH_4 in soils results from the action of methanogen microorganisms that decompose organic material in the absence of O_2 , using CO_2 as an electron acceptor and a reduced organic compound as the donor. The reduction of CO_2 occurs under extended reduced conditions such as in flooded soils or in any soil with severely limited O_2 diffusion [6, 38]. Major factors that influence CH_4 emission flux in soils are soil O_2 , soil CH_4 concentrations, and gas transport. Gas transport is driven mainly by soil water content and temperature [39]. The initiation of CH_4 production is not affected when the dry substrates are stored under dry air, O_2 , or N_2 atmospheres, but it is affected by storage under moist conditions [40]. Therefore, the watering process, in addition to triggering N_2O emission flux, might also have triggered CH_4 and CO_2 production, as shown in Fig. 3g, h. Mikha et al. [15] indicated that after dry soil is watered, dead cells quickly release readily degradable organic compounds, such as amino acids, NH_4^+ compounds, and glycerol, which may be utilized by live microorganisms, resulting in a pulse of CO_2 emission after watering.

Previous studies have reported an inverse correlation between N_2O and CH_4 emissions [4, 41]. Delaune and Reddy [42] report that in soil sediments, anaerobic conditions are reached at redox potential below +400 mv; they also indicated that the approximate range of denitrification activity is between +400 to +300 mv and that the reduction of CO_2 , which yields CH_4 [38, 39], is below -200 mv. Hou et al. [4], in rice paddy soil, and Johnson-Beebout et al. [41], in a rice paddy greenhouse experiment, reported that significant N_2O emissions occurred only at approximated redox potentials above +200 mv, and significant CH_4 occurred below -200 mv. Based on those results, high emissions of both N_2O and CH_4 do not occur simultaneously.

Unlike those studies, this study evaluated the effect of water application to dry beef cattle manure on GHGs. Mayer and Conrad [40] demonstrated that unlike forest and arable soils, rice paddy soils contain a large methanogenic biomass even under dry and aerobic soil conditions, and that the production and emission of CH_4 is limited only by the establishment of low redox potential and the supply of

Table 3 Correlation matrix

	Moist/compacted treatment [†]							
Moist/loose [‡]	CH ₄	N ₂ O	CO ₂	Soil water	Soil temp.	pH	NH ₄ ⁺	NO ₃ ⁻
CH ₄		0.990 (<0.0001)	0.656* (0.039)	+	0.696 (0.025)	+	+	+
N ₂ O	0.990 (<0.0001)		0.552 (0.098)	+	0.734 (0.016)	+	+	+
CO ₂	0.620 (0.042)	0.723 (0.012)		+	+	+	0.576* (0.082)	+
Soil water	0.635 (0.036)	0.711 (0.014)	0.847 (0.001)		+	-0.578* (0.0804)	0.621 (0.055)	-0.806* (0.0049)
Soil temp.	0.596 (0.053)	0.692 (0.018)	0.931 (<0.0001)	0.955 (<0.0001)		+	+	+
pH	-0.601 (0.050)	-0.557 (0.075)	+	+	+		-0.566 (0.088)	0.748* (0.013)
NH ₄ ⁺	0.606 (0.048)	0.681 (0.021)	0.844 (0.001)	0.553 (0.078)	0.694 (0.018)	+		-0.887 (0.0006)
NO ₃ ⁻	-0.688* (0.019)	-0.523 (0.099)	-0.645 (0.032)	-0.745* (0.0085)	-0.561 (0.072)	+	-0.788 (0.004)	

[†] Values above diagonal represent the Pearson Correlation Coefficients and their respective *p* values (in parentheses) for the moist/compacted treatment

[‡] Values below diagonal represent the Pearson Correlation Coefficients and their respective *p* values (in parentheses) for the moist/loose treatment

* No linear relationship was present; instead, a monotonic relationship was observed. Therefore, a Spearman Correlation Coefficient and its *p* value are given, rather than the Pearson Correlation Coefficients

⁺ Empty cells indicate no significant correlation

dissolved organic compounds and oxidants. Moreover, Gattinger et al. [43] reported increased methanogenic biomass in soils with a high rate of manure application. In addition, a soil dominated by air-filled pore space and oxidizing conditions may quickly become saturated with water during recharge events, and reduced conditions and denitrification may dominate temporarily [29]. Therefore, after water application in Experiments 1 and 2, the potentially large amount of aerobic microorganisms present in the dry manure might quickly consume the O₂ left in the substrate and cause a rapid O₂ partial pressure drop [6], thus rapidly activating the likely large population of denitrifiers and methanogens in the dry manure. This result is also supported by the sudden increase in manure temperature after water application (Fig. 3b). Therefore, sudden denitrification and methanogenesis could occur simultaneously as a result of water saturation of the dry manure, which limits O₂ diffusion and enhances microorganism activity.

Table 3 shows that CH₄ emission flux from the moist/loose manure was significantly directly correlated with water content, temperature, and NH₄⁺ content and inversely correlated with pH. It showed a significant monotonic relationship with NO₃⁻ content (Table 3). For the moist/

compacted manure, on the other hand, CH₄ emission flux was significantly correlated only with manure temperature. For CO₂ emission flux, the moist/loose manure showed significant direct correlation between CO₂ emission flux and manure water content, temperature, and NH₄⁺ content and inverse correlation with NO₃⁻ content. Furthermore, the moist/compacted manure showed significant monotonic correlation between CO₂ emission flux and NH₄⁺ content.

Figure 4 shows the temperature trends for the control (dry/loose) and for the moist manure treatments after water application (Experiment 2b). The processes that generated those temperatures differed significantly at a 5 % level of significance. For both moist manure treatments, a quick decrease of 0.5 °C occurred as soon as water was mixed with the manure, which might be a result of direct contact of water with the buried sensors in the manure. After the initial temperature drop, temperature increased (Fig. 4a).

Within the first hour, the moist/loose manure increased 3.9 °C, which is larger than the 3.0 °C observed in the previous experiment (Fig. 3b). This treatment had a net temperature increase of 5 °C 3 h after watering, then dropped to 3 °C at 20 h after watering (Fig. 4b). After this, it observed a second temperature peak, with an increment of 1 °C. These temperature peaks coincided with the peaks

of N_2O and CH_4 emission peaks previously described for the moist/loose manure.

Temperature for the moist/compacted manure exhibited a trend similar to that for the moist/loose manure; however, its maximum registered increment was $2.75\text{ }^\circ\text{C}$, and the respective peak times were different, as shown in Fig. 4a, b. Nevertheless, those peaks also coincided with GHG emission peaks. Field experiments [20] indicated changes in soil/manure temperature of over $9\text{ }^\circ\text{C}$ between different surface conditions within a pen in a beef cattle feedlot. In general, results shown in Fig. 4 confirm results from previous experiments, and the temperature trends support the GHG emission peaks reported in this study.

Thirty-five days after first watering, a second watering event took place. As shown in Fig. 4a, a new set of temperature peaks was observed, but those peaks did not reach previous levels. This result might be a consequence of NH_4^+ depletion because no new urine or manure was added in this experiment. That NH_4^+ depletion might result in low nitrification activity in the manure, which would decrease denitrification; therefore, those small temperature peaks might be result of inhibited microorganism activity. Because the N inputs as urine and manure on a pen surface can be considered inexhaustible in an open-lot beef cattle feedlot, it might be suggested that large emission peaks of GHGs are emitted after each rainfall event on dry soil/manure surfaces.

Conclusions

This study evaluated the effects of water application on GHG emission fluxes from beef cattle feedlot manure. The following conclusions can be drawn:

1. Emission fluxes of GHGs from dry/loose manure were significantly smaller than those from moist manure. As soon as 10 min after water application on the dry manure, large peaks of emission fluxes were observed. Emission flux peaks for the moist/compacted manure were significantly smaller than those for the moist/loose manure. Both the moist/loose and the moist/compacted manure showed a second set of GHG emission peaks, which were lower than the first peaks, a few days after water application.
2. A large but short-term denitrification occurred within 10 min after water application on dry soil/manure, which might be responsible for the large GHG emission fluxes.
3. When the manure dried and with no additional inputs of urine, feces, or water, the GHG emission fluxes decreased to the level for dry/loose manure.
4. For the moist/loose manure, direct significant correlation was found among N_2O , CH_4 , and CO_2 emission fluxes with water content, temperature, and NH_4^+ content;

significant but inverse correlation also was observed between those GHGs and manure pH and NO_3^- content.

5. For the moist/compacted manure, N_2O and CH_4 emission fluxes showed significant direct correlation only with manure temperature.

Results suggest that in estimating emission fluxes from soil/manure, the effect of water content and/or water application should be considered. Also, water sprinkling for controlling dust emission and/or alleviating heat stress on the animals could increase greenhouse gas emissions from pen surfaces, and should therefore be considered when designing or operating water sprinkler systems.

Acknowledgments This study was supported in part by the government of the Republic of Panama through SENACYT/IFARHU/ Universidad Tecnológica de Panamá, Kansas Agricultural Experiment Station (contribution number 14-207-J), and USDA-NIFA Special Research Grant “Air Quality: Reducing Air Emissions from Cattle Feedlots and Dairies (TX and KS),” through Texas AgriLife Research.

Conflict of interest The authors declare that they have no competing interests.

Author’s contributions OAA designed the experiments, with inputs from co-authors, collected and analyzed the data, and prepared the initial draft of the manuscript. RGM conceived the study, provided inputs on the experimental design and analysis of data, and made critical revisions to the manuscript. SLT provided inputs in measurement, provided measurement instrumentation, and contributed to the manuscript. LEE provided input in the experimental design, analysis and interpretation of data, and manuscript. All authors reviewed and approved the final manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Intergovernmental Panel on Climate Change, Fourth Assessment Report: Working group I: The Physical Science Basis. (2007). http://www.ipcc.ch/publications_and_data/ar4/wg1/en/spmssp-human-and.html. Accessed 20 August 2012
2. Raupach, M., Fraser, P.: Chapter 2: climate and greenhouse gases. CSIRO, Australia. <http://www.csiro.au/Outcomes/Climate/Climate-Change-Book/Chapter-2-Climate-and-greenhouse-gases.aspx>. (2013). Accessed 20 August 2013
3. Stackhouse, K.R., Pan, Y., Zhao, Y., Mitloehner, F.M.: Greenhouse gas and alcohol emissions from feedlot steers and calves. *J. Environ. Qual.* **40**, 899–906 (2011)
4. Hou, A.X., Chen, G.X., Wang, Z.P., Van Cleemput, O., Patrick, W.H.: Methane and nitrous oxide emissions from a rice field in relation to soil redox and microbiological processes. *Soil Sci. Soc. Am. J.* **64**, 2180–2186 (2000)
5. Li, C., Salas, W., Zhang, R., Krauter, C., Rotz, A., Mitloehner, F.: Manure-DNDC: a biogeochemical process model for quantifying greenhouse gas and ammonia emissions from livestock manure systems. *Nutr. Cycl. Agroecosyst.* **93**(2), 163–200 (2012)

6. Li, C.: Quantifying greenhouse gas emissions from soils: scientific basis and modeling approach. *Soil Sci. Plant Nutr.* **53**, 344–352 (2007)
7. National Research Council (NRC): *In situ bioremediation-When does it work?* The National Academies Press, Washington DC (1993)
8. Hofstra, N., Bouwman, A.F.: Denitrification in agricultural soils: summarizing published data and estimating global annual rates. *Nutr. Cycl. Agroecosyst.* **72**, 267–278 (2005)
9. Healy, R.W., Striegl, R.G., Russel, T.F., Hutchinson, G.L., Livingston, G.P.: Numerical evaluation of static-chamber measurements of soil-atmosphere gas exchange-identification of physical processes. *Soil Sci. Soc. Am. J.* **60**, 740–747 (1996)
10. Parkin, T.B., Kaspar, T.C.: Nitrous oxide emissions from corn-soybean systems in the Midwest. *J. Environ. Qual.* **35**, 1496–1506 (2006)
11. Kanako, K., Ronggui, H.U., Sawamoto, T., Hatano, R.: Three years of nitrous oxide and nitric oxide emissions from silandic andosols cultivated with maize in Hokkaido. *Jpn. Soil Sci. Plant Nutr.* **52**, 103–113 (2006)
12. Davidson, E.A.: Sources of nitric-oxide and nitrous-oxide following wetting of dry soil. *Soil Sci. Soc. Am. J.* **56**(1), 95–102 (1992)
13. Scholes, M.C., Martin, R., Scholes, R.J., Parsons, D., Winstead, E.: NO and N₂O emissions from savanna soils following the first simulated rains of the season. *Nutr. Cycl. Agroecosyst.* **48**(1–2), 115–122 (1997)
14. Ellert, B.H., Janzen, H.H.: Nitrous oxide, carbon dioxide and methane emissions from irrigated cropping systems as influenced by legumes, manure and fertilizer. *Can. J. Soil Sci.* **88**, 207–217 (2008)
15. Mikha, M.M., Rice, C.W., Milliken, G.A.: Carbon and nitrogen mineralization as affected by drying and wetting cycles. *Soil Biol. Biochem.* **37**, 339–347 (2005)
16. De Klein, C.A., McTaggart, P., Smith, K.A., Stevens, R.J., Harrison, R., Laughlin, R.J.: Measurement of nitrous oxide emissions from grassland soil using photo-acoustic infra-red spectroscopy, long-path infra-red spectroscopy, gas chromatography, and continuous flow isotope-ratio mass spectrometry. *Commun. Soil Sci. Plan.* **30**(9–10), 1463–1477 (1999)
17. Guo, L., Maghirang, R.G., Razote, E.B., Auvermann, B.W.: Laboratory evaluation of dust-control effectiveness of pen surface treatments for cattle feedlots. *J. Environ. Qual.* **40**, 1503–1509 (2011)
18. Razote, E.B., Maghirang, R.G., Predicala, B.Z., Murphy, J.P., Auvermann, B.W., Harner III, J.P., Hargrove, W.L.: Laboratory evaluation of the dust-emission potential of cattle feedlot surfaces. *T. ASABE.* **49**(4), 1117–1124 (2006)
19. Morrow, J.L., Mitloehner, F.M., Johnson, A.K., Galyean, M.L., Dailey, J.W., Edrington, T.S., Anderson, R.C., Genovese, K.J., Poole, T.L., Duke, S.E., Callaway, T.R.: Effect of water sprinkling on incidence of zoonotic pathogens in feedlot cattle. *J. Anim. Sci.* **83**, 1959–1966 (2005)
20. Aguilar, O.A., Maghirang, R., Rice, C.W., Trabue, S.L., Erickson, L.E.: Nitrous oxide fluxes from a commercial beef cattle feedlot in Kansas. *Air Soil Water Res.* **7**, 35–45 (2014). doi:10.4137/ASWR.S12841
21. Chiyoka, W.L., Hao, X., Zvomuya, F., Li, X.: Nitrous oxide emissions from chernozemic soils amended with anaerobically digested beef cattle feedlot manure: a laboratory study. *Anim. Feed Sci. Technol.* **166–67**, 492–502 (2011)
22. Aguilar, O.A., Maghirang, R., Rice, C.W., Trabue, S.L., Erickson, L.E.: Laboratory evaluation of surface amendments for controlling greenhouse gas emissions from beef cattle feedlots. *IJEE.* **4**, 41 (2013)
23. Milliken, G.A., Johnson, D.E.: *Analysis of Messy Data vol. 1: Designed Experiments.* Taylor & Francis Group, New York (2009)
24. SAS Institute, *SAS/STAT 9.2 User's guide*, 9.2 (2008)
25. R Project: Ver. 2.15.1 (Roasted Marshmallows). The R Project for Statistical Computing, Nashville (2012)
26. Kanako, K., Ronggui, H.U., Sawamoto, T., Hatano, R.: Comparison of the closed-chamber and gas concentration gradient methods for measurement of CO₂ and N₂O fluxes in two upland field soils. *Soil Sci. Plant Nutr.* **54**, 777–785 (2008)
27. Kanako, K., Sawamoto, T., Hatano, R.: Nitrous oxide emissions for 6 years from a gray lowland soil cultivated with onions in Hokkaido. *Jpn. Nutr. Cycl. Agroecosyst.* **63**, 239–247 (2002)
28. Marinho, E.V.A., Delaune, R.D., Lindau, C.W.: Nitrous oxide flux from soybeans grown on Mississippi alluvial soil. *Commun. Soil Sci. Plan.* **35**(1), 1–8 (2004)
29. National Research Council (NRC): *Soil and Water Quality: an Agenda for Agriculture.* The National Academies Press, Washington, DC (1993)
30. Sagar, S., Andrew, R.M., Tate, K.R., Hedley, C.B., Rodda, N.J., Townsend, J.A.: Modelling nitrous oxide emissions from dairy-grazed pastures. *Nutr. Cycl. Agroecosyst.* **68**, 243–255 (2004)
31. Groffman, P.M., Rice, C.W., Tiedje, J.M.: Denitrification in a tallgrass prairie landscape. *Ecology* **74**(3), 855–862 (1993)
32. Intergovernmental Panel on Climate Change. *IPCC Guidelines for National Greenhouse Gas Inventories*, Chap. 4, Agriculture (1996). <http://www.ipcc-nggip.iges.or.jp/public/gl/invs6c.html>. Accessed 20 August 2012
33. Klein, C.A.M., Logtestijn, R.S.P.: Denitrification and N₂O emission from urine-affected grassland soil. *Plant Soil* **163**(2), 235–241 (1994)
34. Lee, J., Hopmans, J.W., van Kessel, C., King, A.P., Evatt, K.J., Louie, D., Rolston, D.E., Six, J.: Tillage and seasonal emissions of CO₂, N₂O, and NO across a seed bed and at the field scale in a Mediterranean climate. *Agr. Ecosyst. Environ.* **129**, 378–390 (2008)
35. Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., Van Cleemput, O.: Closing the global N₂O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutr. Cycl. Agroecosyst.* **52**, 225–248 (1998)
36. Woodbury, B.L., Miller, D.N., Nienaber, J.A., Eigenberg, R.A.: Seasonal and spatial variations of denitrifying enzyme activity in feedlot soil. *T. ASABE.* **44**, 1635–1642 (2001)
37. Taghizadeh-Toosi, A., Clough, T.J., Condron, L.M., Sherlock, R.R., Anderson, C.R., Craigie, R.A.: Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. *J. Environ. Qual.* **40**, 468–476 (2011)
38. Paul, E.A. (ed.): *Soil Microbiology, Ecology, and Biochemistry.* Academic Press, Burlington (2007)
39. Segers, R.: Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry* **41**, 23–51 (1998)
40. Mayer, H.P., Conrad, R.: Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. *FEMS Microbiol. Ecol.* **73**(2), 103–111 (1990)
41. Johnson-Beebout, S.E., Angeles, O.R., Alberto, M.C., Buresh, R.J.: Simultaneous minimization of nitrous oxide and methane emission from rice paddy soils is improbable due to redox potential changes with depth in a greenhouse experiment without plants. *Geoderma Sci. Direct.* **149**, 45–53 (2008)
42. Delaune, R.D., Reddy, K.R.: *Redox Potential.* University of Florida. Elsevier Ltd., Gainesville (2005)
43. Gattinger, A., Hoeffle, M.G., Schloter, M., Embacher, A., Bohme, F., Munch, J.C., Labrenz, M.: Traditional cattle manure application determines abundance, diversity and activity of methanogenic Archaea in arable European soil. *Environ. Microbiol.* **9**(3), 612–624 (2007)