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Strategies for Reducing Greenhouse Gases from Liquid Dairy Manure

Vera Sokolov
bosa4400@mylaurier.ca

Strategies for Reducing Greenhouse Gases from Liquid Dairy Manure

by

Vera Katerina Sokolov

DISSERTATION

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Livestock production, including the storage, handling, and spreading of manure, are among the largest contributors to greenhouse gas emissions from the agricultural sector. Liquid dairy manure storages are hot spots of methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3). Both CH_4 and N_2O are greenhouse gases (GHG) which contribute to global warming, while NH_3 is an indirect source of N_2O and a risk to human health. Reducing emissions from manure storages is important not only for protection of environment and humans, but also for conserving the nutrients in manure making it valuable as a fertilizer. This thesis contributed to the advancement of GHG reducing strategies for liquid dairy manure by: i) testing gradual and batch fillings methods with inoculum stored manure ii) field-scale and lab-scale studies of dairy manure acidification, and iii) a quantitative and qualitative review of 12 years of research from a meso-scale manure storage facility. Gradually-filled and batch-filled meso-scale manure tanks with inoculum (0%, 10% or 20%) were compared on their GHG emissions. On average, gradually-filled tanks had 1.4°C higher manure temperature, which may have contributed to a 12% increase in total CH_4 (6.26 kg m^{-3}) and 28% increase in NH_3 emissions (358 g m^{-3}). The 10% and 20% inoculum tanks produced comparable emissions, while the 0% tanks (4.84 kg m^{-3}) produced markedly lower CH_4 (24%). Acidification using H_2SO_4 was explored at different rates of application, with or without inoculum, in a laboratory incubation and in meso-scale storages. The novelty of this research was reducing the frequency of acidification, acidifying only once throughout the storage period and an overall focus on reducing cost. Acidification had up to 89% CH_4 reduction and 53% NH_3 reductions using 1.1 – 2.4 mL acid L^{-1} manure. In laboratory incubations, H_2SO_4 reduced CH_4 production by 80% at 17°C, 90% at 20°C, and 19% at 23°C. Results also indicated that residual slurries of acidified manure were a poor inoculant in subsequent storage periods, hence manure acidification reduced CH_4 for two fill-empty cycles. Lastly, analysis of meso-scale trials (2006-18) compared treatment differences using Cohen's d effect size. Manure acidification had the largest effect size (up to 6.03) compared to using manure covers, inoculum removal, and dilution which had effect sizes as low as 0.096. Overall, this thesis contributed to the advancement of reducing GHG emissions from liquid dairy manure through original research by: i) highlighting the bias in batch-filling experimental storages ii) creating strategies for reducing cost of acidification while retaining good treatment effects iii) compared GHG reducing strategies from over a decade of research, highlighting acidification as having the best treatment potential.

Vera Katerina Sokolov

Wilfrid Laurier University, 2020

Advisor:

Jason Venkiteswaran

To my grandmother, Marie Bosak.

A brave and inspiring woman.

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List of Abbreviations

Methane	CH_4	Particulate Matter	PM
Nitrous Oxide	N_2O	Standard Deviation	SD
Ammonia	NH_3	Mean Squared Error	MSE
Greenhouse Gas	GHG	Total Methane Production	G_o
Bioenvironmental Engineering Centre	BEEC	Degrees of Freedom	df
		F-value	F
Fresh Manure	FM	p-value	p
Dry Matter	DM	eta-squared	η_P^2
Total Solids	TS	90% Confidence Interval	CI_{90}
Volatile Solids	VS	Lag Phase	λ
Volatile Fatty Acid	VFA	Maximum Daily Methane Production	R_{max}
Organic matter	OM		
Nitrogen	N	Sulfuric Acid	H_2SO_4
Nitrite	NO_3	Newly Acidified	NA
Carbon Dioxide	CO_2	Previously Acidified	PA
Total Nitrogen	TN	Inoculum	Inoc
Total Ammonium-N	TAN	Number	N

List of Units

mg	Milligram	g	Gram
kg	Kilogram	m	Meter
L	Liters	cm	Centimeter
mL	Milliliter	$	Canadian Dollar
m^3	Cube Meter	min	Minute
lb	US Pound	µg	Microgram
gal	US Gallon	s	Second
d	Day	nm	Nanometers
mon	Month	µm	Micrometers
t	Ton	y	Year
week	Week	h	Hour
˚C	Degrees Celsius	CO_2-eq	CO_2 equivalent
%	Percent	Mt	Mega tonne
m^2	Square Meter	kt	Kilotonne
ppm	Part Per Million	mol	moles

1. Introduction

Liquid diary manure is a large source of environmentally problematic greenhouse gases (GHG), predominantly methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3). Both CH_4 and N_2O are greenhouse gases (GHG) which contribute towards global warming, while NH_3 is a toxic gas which contributes to city smog and environmental acidification. N_2O is a more powerful greenhouse gas, with a global warming potential of 298 times that of carbon dioxide (CO_2). However, CH_4 is the predominant gas emitted from liquid manure, even on a CO_2 equivalent bases, with its global warming potential being 34. The nature of manure is such, that it is both a commodity and a nuisance. It is a waste product from dairy production, however, it is also a powerful fertilizer for agricultural crops. During storage, transport, and field spreading, nutrients can be lost from manure through gaseous emissions. This reduces the value of the manure and increases biogenic gases in the atmosphere. In managing manure, we must therefore aim for two goals: a) protect the environment by reducing GHG emissions, and b) preserve the nutrients in the manure and consequently its value as a fertilizer. Additionally, reducing GHG emissions from dairy manure is increasingly important in Canada, as the increasing population puts production demands on the dairy industry, and carbon taxing along with consumer opinion push for sustainable production.

Storing manure as a liquid, rather than solid, is a popular option for farmers as it allows for concurrent barn wash water and bedding disposal and is easily transported away from the barn into storages and ultimately onto fields. Manure tanks and other storages are continuously filled throughout the year but are only emptied only once or twice a year during field spreading. This creates a point source of GHG emissions to the atmosphere. The benefit of a point source emitter, is that treatments and practices can be implemented for reducing GHG emissions.

In order to reduce GHG emissions from manures storage, GHGs can be trapped and converted into other forms, or they can be prevented by making the environment inhospitable for microbial activity. Trapping biogas is done with covers or specialised equipment, such as an anaerobic digester. Natural and synthetic cover have shown limited ability to reduce GHGs, often creating trade-offs of decreasing CH_4 while increasing N_2O (Alexander 1977; Owen and Silver 2015). Anaerobic digestion is a way of utilizing biogas for electricity production, however the start-up cost is often prohibitive. A common practice limiting microbial growth is solid-liquid separation, where the solids containing methanogen substrates are removed from the liquid (Møller et al. 2004a). Although high CH_4 production can be achieved in the liquid portion, unless the solids are well dried there is possibility of increased N_2O production (Alexander 1977). Acidification and inoculum removal both limit microbial growth in manure storages. Acidification can reduce NH_3 volatilization by shifting the equilibrium of ammonium (NH_4^+) and NH_3 to NH_4^+ and hence keeping it in solution (Clemens et al. 2002). Additionally, low the pH may disrupt methanogenesis and reduce CH_4 production. Inoculum removal, on the other hand, removes inoculating methanogens and hence new methanogen colonies need to establish in the manure storage which increases the lag time for CH_4 emissions, as well as overall emissions.

The aim of this thesis is to develop strategies for mitigating GHG emissions from liquid dairy manure storages. This was done by exploring the relationship of inoculum presence and gradual versus batch filling and quantifying the GHG reductions using manure acidification and inoculum acidification. The research was conducted at the Bio-Environmental Engineering Centre (BEEC) which has been used for over a dozen experiments investigating methods for emission reductions from manure storages. The results from these experiments were synthesized for a comparative analysis to highlight the best strategies for reducing GHG emissions.

1.1. Inoculum and Gradual Filling

When manure tanks are emptied, often once or twice a year, it is difficult for farmers to fully remove all the manure and clean the tank. Aged residual manure at the bottom of manure storage tanks remains. This manure has established microbial colonies which are acclimated to the manure environment and hence thrive in it. Once fresh manure is added, the microbes quickly start to propagate as they encounter fresh substrate. However, if there is no aged manure, then microbial colonies in fresh manure need to acclimate and hence cause a lag in production and in total produce fewer GHGs over the storage period. Research into the effect of residual manure/inoculum, has so far been done by one-time batch filling meso-scale storages or laboratory incubation jars with fresh manure and 5 – 50% inoculum (Massé et al. 2003; Møller et al. 2004b; VanderZaag et al. 2010a, 2017; Wood et al. 2014a; Ngwabie et al. 2016; Le Riche et al. 2017; Baral et al. 2018). However, on dairy farms, tanks are filled on a daily or weekly basis. Batch filling storages containing residual manure may change the GHG emissions by changing the ratio of substrate (fresh manure) to microorganisms (inoculum) and cause an accumulation of acidic compounds which are toxic to methanogens (Lyberatos and Skiadas 1999). This change in filling type may be an unaccounted variable and needs to be explored. This research may help us understand how meso-scale GHG emissions compares to on-farm manure storage GHG emissions and inform future research on best practices.

1.2. Acidification

Acidification of liquid manure is a promising method for reducing GHG emissions. Reducing manure pH changes the environment, forcing methanogens to acclimatize and in response produce fewer emissions over the storage period. Methanogens may be directly harmed by the acid or be forced to go dormant during unfavorable conditions. As such, acid may be directly

applied to fresh manure or be used in lieu of storage cleaning, as it may disrupt the inoculating effect of the residual manure.

Research into manure acidification has been done predominantly on pig slurry in European countries to reduce NH_3 emissions. Previous research has reported CH_4 emissions reductions of 17 – 90% and NH_3 reductions of 40 – 98%, depending on animal slurry and acid type used (Lefcourt and Meisinger, 2001; Shi et al., 2001; Berg et al., 2006b; Kai et al., 2008; Wang et al., 2014; Fangueiro et al., 2015). As it may prove to be an effective practice for reducing GHG emissions on Canadian dairy farms, there is need to fully research acidification in a Canadian setting. Results from other countries may not be transferrable as differences in housing, feed, farm practices, and climate can all influence the GHG emissions and treatment responses. No research has explored dairy manure acidification in Canada. Before doing on-farm trials, however, there is need to test different rates of acidification in laboratory incubations and meso-scale field trials. The research in this thesis will set the groundwork for acidification practises in Canada by informing best acid rates and methods of application.

1.3. 10-year Data

For >10 years BEEC at the Dalhousie University Agricultural Campus has been used for studying GHG emissions from experimentally sized, meso-scale manure tanks, with the purpose of finding strategies that mitigate GHG production and emissions. Each manure tank contains ~10 m^3 of manure and are monitored continuously for CH_4, N_2O, and NH_3. Treatments which have been studied include: natural and synthetic covers, reducing volatile solids in manure by dilution or changing bedding type, and inoculum removal. Although many of these treatments have found positive results, there are often drawbacks or practical issues which make the treatment not feasible. Straw covers reduced CH_4 emissions by 24% and NH_3 by 84%, but also

correspondingly increased N_2O emissions by 60% (VanderZaag et al. 2009). Synthetic covers, on the other hand, reduced NH_3 fluxes by 90% and N_2O by 70%, but had no effect on CH_4 (VanderZaag et al. 2010b). Diluting manure showed an impressive 52% reduction in CH_4 emission per area, however this corresponded to a 95% increase of CH_4 when scaled by volatile solids (VS; g kg VS^{-1}) (Wood et al. 2012). Using non-organic sand bedding instead of degradable sawdust bedding reduced CH_4 emissions by 33% and N_2O by 85%, but increased NH_3 by 10% (Le Riche et al. 2017). Lastly, inoculum removal had promising results with up to ~50% CH_4 reductions when tanks were completely cleaned (compared to 5-50% inoculum) prior to addition of fresh manure (Wood et al. 2014a). However, completely removing manure from storage tanks is not always practically feasible on farms.

An analysis of all dataset collected at this site since 2006 may provide insight into relationships of GHG emissions and variables such as TS/VS, temperature, and inoculum. Additionally, it may allow us to compare treatments that were studied for more than one year and highlight the best strategies for reducing emissions. This dataset spans >10 years of research allowing for comparison of different manure GHG mitigating practices which can inform best management practices for farmers and policy makers.

1.4. Research Objectives

This research aims to build upon the existing research on mitigating GHG from stored liquid dairy manure by testing mitigation practices in unique ways, testing new practices, and synthesizing results to make overall recommendations. The main objectives of this research were:

I. Compare the impact of gradual and batch tank filling at different inoculum level on GHG and NH_3 emissions and relate them to manure properties.

II. Monitor and assess the impact of fresh manure with untreated 6 m old inoculum, acidified 6 m old inoculum, newly acidified 6 m old inoculum, and no inoculum, and of acidified fresh manure with untreated 6 m old inoculum, incubated at 17, 20, and 23°C on CH_4 and CO_2 production.

III. Quantify changes of acidifying fresh manure at 3 different rates on GHG and NH_3 emissions and relate them to manure properties.

IV. Compare the impact of fresh manure with 12 m old untreated inoculum, 12 m old acidified inoculum, or fresh manure with 12 m old untreated inoculum on GHG and NH_3 emissions and relate them to manure properties and bacterial and methanogen colonies.

V. Integrate research results from >10 years of GHG and NH_3 emissions measurements from a meso-scale manure storage facility and compare strategies for reducing emissions.

1.5. Significance of Work

This research enhanced our understanding of using meso-scale storage system for studying GHG emissions from liquid dairy manure by comparing gradual and batch filling. Although meso-scale systems are used for comparison between treatments, understanding how closely they represent on-farm tanks is important for upscaling research to on-farm system and general understanding of processes occurring in manure. It also deepened our understanding of the effect residual manure (inoculum) has on GHG production which can inform future research and management practices. The acidification work in this thesis is the first steps in evaluating this treatment for Canada. Specifically, this thesis explored possible rates of application, methods of application, and the most cost-effective way to treat manure, including lower or fewer application rates and acidifying only inoculum instead of all fresh manure. Lastly, analysis of 12

season-long studies from BEEC reviews results and methods, uncovers trends in data, defines treatment effects between years, and lastly, summarizes the main results and recommendations from >10y of storage emission research.

2. Background

2.1. Greenhouse Gases and Dairy Manure in Canada

The Canadian population is projected to increase from 35.2 million to 51 million people in the next 50 years, which in turn will increase demands on dairy and meat production (Statistics Canada 2014). As of 2017, Canada has 10,593 dairy farms, with 1.41 million dairy cows and heifers producing 89.8 million hectoliters of milk annually (Canadian Dairy Information Centre 2017). An average Canadian dairy farm has between 89 and 133 cows and heifers (Canadian Dairy Information Centre 2017; Agriculture and Agri-Food Canada and Canadian Dairy Information Centre 2019).

In the last 100 years, Canadian agriculture has shifted to fewer and larger farms, resulting in larger amounts of manure being created at each facility (Agriculture and Agri-Food Canada 2016). Dairy cows produce approximately 2 – 3 m^3 $head^{-1}$ d^{-1} of liquid manure or ~100,000 m^3 $farm^{-1}$ y^{-1} (British Columbia Ministry of Agriculture 2015). Since manure field application is allowed only during the growing season (to minimize winter run-off and surface pollution), while manure is created continuously, farms need infrastructure for storing and managing manure. This results in hotspots of GHG emissions.

Canada contributes about 1.6% to the world's total GHG emissions, which is approximately 722 megatonnes (Mt) carbon dioxide equivalent (CO_2-eq) emissions (Government of Canada 2012; Environment and Climate Change Canada 2017a). As a comparison, China contributes about 25.9%, and the United States about 14.0% to the world's total GHG emissions (Government of Canada 2012). Although it may seem that Canada contributes very little on a global scale, it is important to note that China's population is 18.5% of the world's, while United States' is 4.3% while Canada's is only 0.48% (United Nations Department of Economic and Social Affairs 2017). This means that on a per capita basis, Canadian GHG emissions are more than double that of China.

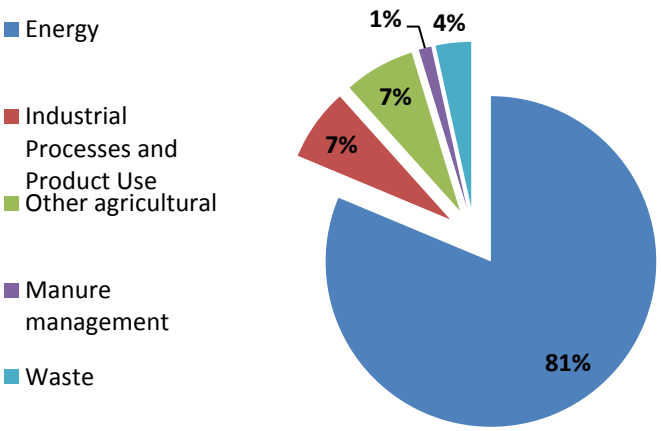

Figure 2.1 Canadian greenhouse gas emissions (%) from manure management within the context of emissions from all other sectors.

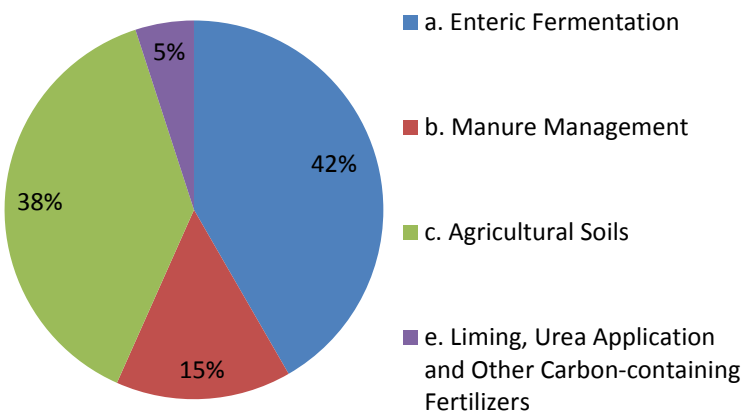

Figure 2.2 Breakdown of annual Canadian greenhouse gas emissions from the agricultural sector, expressed as percent (%) CO_2 equivalent emissions (CO_2-eq) (Environment and Climate Change Canada, 2017).

Within Canada, approximately 8% of GHG emissions are sourced from the agricultural sector (Government of Canada 2012). In 2016, this was 59 Mt of CO_2-eq GHG emissions. Of this, 9 Mt of CO_2-eq GHGs were from manure management, which is 15% of all Canadian agricultural emissions and 1% of all Canadian emissions (Figure 2.1**Figure 2.1 Canadian greenhouse gas emissions (%) from manure management within the context of emissions from all other sectors.** and Figure 2.2). The largest agricultural emissions come from enteric fermentation and agricultural soil, which together make 80% of agricultural GHG emissions.

The Canadian National Inventory Report submitted to the United Nations separates agricultural emissions into enteric, manure management, and field sourced. Dairy cattle were reported to have annual emissions of 4,920 kilotonnes (kt) CO_2-eq, with 75% of the emissions from enteric fermentation and 25% from manure management. The predominant GHG emitted from both dairy cattle sources was CH_4 (92%), with enteric fermentation emitting exclusively CH_4. Manure

management from dairy cattle emitted 68% CH_4 and 32% N_2O (not accounting for indirect sources of N_2O).

2.2. Greenhouse gas production

Manure is a unique environment, which is mostly liquid (>75% water) and yet high in organically degradable compounds (~900 g biochemical oxygen demand day^{-1} for dairy cows; ~135 g biochemical oxygen demand day^{-1} for swine) (Agriculture and Agri-Food Canada 1980). During animal digestion, 60-90% of nutrients (potassium, nitrogen, and phosphorus) from ingested feed are excreted (Agriculture and Agri-Food Canada 1980). The organic matter (OM) in manure is partially digested and in various forms of decomposition. While some compounds will be in the form of volatile fatty acids (VFAs), which are readily converted to CH_4, others still need to undergo hydrolysis which breaks down large organic polymers. It is important to understand the processes of methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3) production, to understand how to mitigate them.

2.2.1. Hydrolysis

The first phase of OM degradation is hydrolysis, which is an extracellular, enzymatic process breaking particulate carbohydrates, proteins, and lipids into simple sugars, amino acids, and fatty acids (Lyberatos and Skiadas 1999; Mao et al. 2015) (Figure 2.3). Following hydrolysis (also called ammonification), nitrogen is released in the form of ammonia (Vavilin et al. 2008). Hydrolysis is considered the rate-limiting step in anaerobic digestion, as acidogenesis and acetogenesis, which follow, are rapid reactions (Vavilin et al. 2008). Acidogenesis is an intracellular process which creates VFAs (ex: propionic acid, butyric acid, acetic acid, H_2, and CO_2) and alcohols (Figure 3.4.) (Lyberatos and Skiadas 1999). Lastly, acetogenesis, hydrogenesis, and homoacetogenesis create acetic acid, H_2, and CO_2 (Figure 2.3). Methane production will occur under the right environmental conditions using acetic acid, H_2, and CO_2. It

is important to understand OM degradation processes, especially hydrolysis, because mitigation techniques that can reduce hydrolysis, will also reduce CH_4, NH_3, and N_2O production. The digestibility of feed and amount of bedding in the manure will determine how much OM is available for hydrolysis.

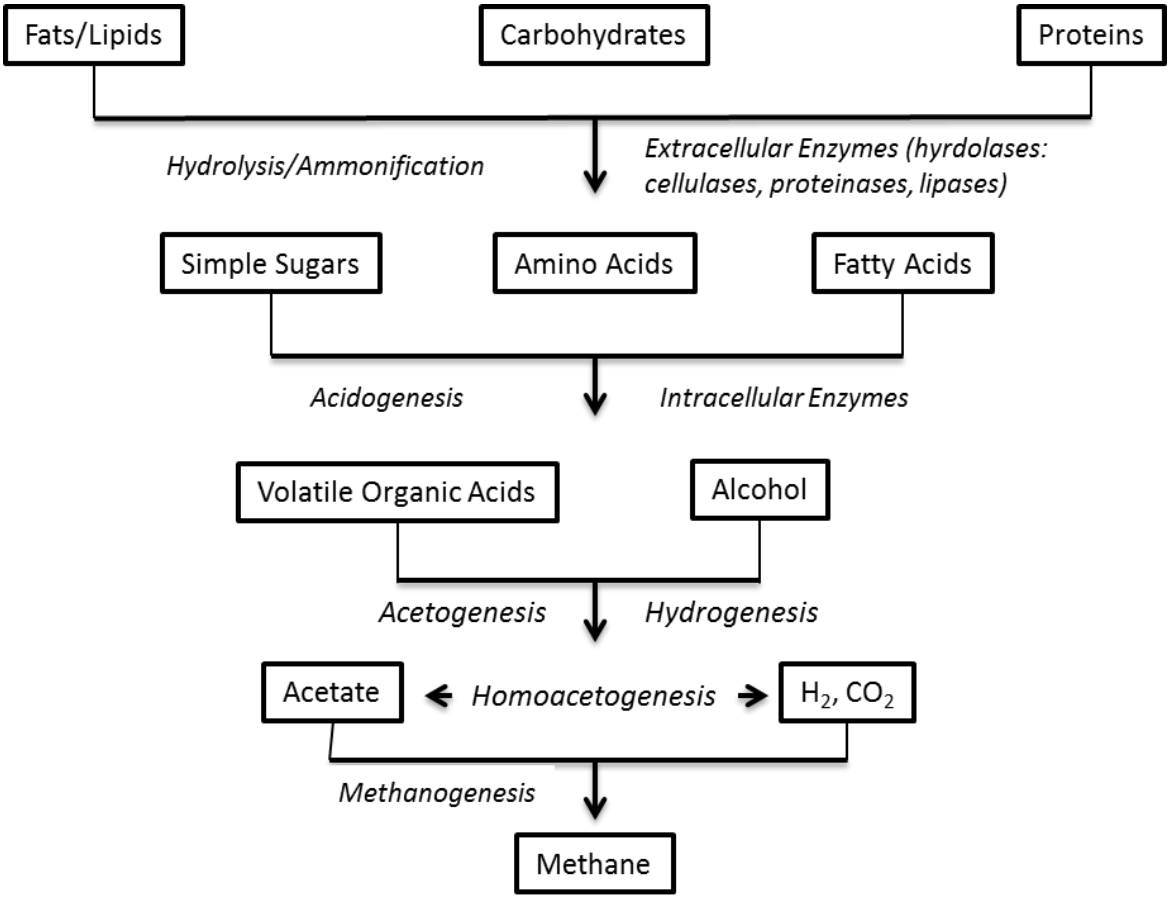

Figure 2.3 Biochemical breakdown of organic matter during anaerobic digestion. Modified from Lyberator and Skiadas (1999) and Mao et al. (2015).

2.2.2. Methanogenesis

Methanogenesis is a strictly anaerobic process that creates CH_4 by methanogenic microorganisms. There are 4 different reaction pathways known to result in CH_4 production by microorganisms (Ferry 2010):

$$\boldsymbol{CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O} \qquad \text{Eq. 2.1}$$

$$4HCO_2H \rightarrow 3CO_2 + CH_4 \quad \text{Eq. 2.2}$$

$$4CO + 2H_2O \rightarrow 3CO_2 + CH_4 \quad \text{Eq. 2.3}$$

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad \text{Eq. 2.4}$$

Although there are many factors that affect methanogenesis, we will discuss only the main four: temperature, substrate/VS, pH, and inoculum.

2.2.3. Temperature

The temperature range in which methanogens can function in is 5-70℃. However, specific methanogen species will usually fall into one of three temperature ranges: Thermophilic (55-70℃), Mesophilic (~37℃), or Psychrophilic (5-20℃) (Mao et al. 2015). With increasing temperature, (thermophilic compared to mesophilic ranges), the productivity of methanogens increases by having faster reaction rates and higher load bearing capacity (Mao et al. 2015). In fact, many studies have observed a clear positive relationship with temperature increase with GHG production (Massé et al. 2003, 2008; Wood et al. 2013). However, there are many aspects of the temperature and CH_4 production relationship that are not well understood. The relationship of CH_4 production with temperature does not seem to be a simple one, especially since other factors or treatments can affect the temperature response. For example, Blake et al. (2015) studied the response of methanogens in arctic sediment to temperature and substrate availability. Changing the substrate would result in growth of different methanogen strains by promoting one or more CH_4 producing reaction (Eq. 2.6 – 2.9). They found that with change in substrate the temperature dependency of CH_4 production changed (Blake et al. 2015). In fact, the methanol amended treatment had very little change, but highest CH_4 production at 20 ℃. Considering the variability and complexity of manure, it is difficult to predict temperature response.

There is a knowledge gap in the temperature response of methanogens at temperatures below 25℃. As most CH_4 production research is done on anaerobic digestion, little work has been done at lower temperatures. Massé et al (2003, 2008) measured CH_4 emissions from various slurry types at 10°C and 15°C, and 10°C and 20°C. In both studies, they found that methane production was substantially greater at higher temperatures, however, there is need for studies with smaller increments of temperature changes to fully describe the temperature relationship. Lastly, there is need for research to fully quantify CH_4 production with short-term (e.g. hourly or daily) temperature fluctuations, such as might be seen on-farm for better predictive power and possible mitigation strategies.

2.2.4. Substrate/VS

Many researchers agree that methanogenesis follows the Monod kinetic function, which relates microbial growth to the limiting substrates in the environment (Lyberatos and Skiadas 1999). In the standard Monod relationship curve, increasing substrate increases CH_4 until it reaches a plateau (Monod 1949). The substrates for methanogenesis are often quantified as VFAs, but most frequently as VS. VS are assumed to be a representative measure of OM, but are any material that volatilises between 100-500℃. VFAs are a more precise measure of direct substrate availability for methanogens, since they are directly converted to CH_4 through acetogenesis and methanogenesis. However, VFAs represent only the currently available substrates, rather than all that will be available over the course of decomposition. Substrate affects CH_4 production in several ways, starting with amount present. As per the Monod function, decreased substrate means decreased CH_4 production. Another is the retention time. In terms of manure management, the longer the methanogens are exposed to the substrate (in the right environment), the more CH_4 will be produced (Nges and Liu 2010). A change in VS/substrate also makes a difference, as a large amount of substrate added can dramatically alter the environment and

hinder CH_4 production (Mao et al. 2015). Inflow of substrates can increase other reactions, such as hydrolysis and acidogenesis, which in turn cause VFA accumulation and disruption of CH_4 production through acidification (Mao et al. 2015). Lastly, a low C:N ratio can increase ammonia production, which can hinder CH_4 production (Mao et al. 2015).

2.2.5. pH

The pH range for methanogenesis is 6.5-8.2, with pH 7 being optimal (Mao et al. 2015). Within manure different reactions act to increase or decrease pH. Acidogenesis, which creates substrate for methanogens, is optimal at 5.5-6.5 (Mao et al. 2015). This means there is only a small range of optimal pH where methanogens receive substrate and are able to use it. Acidogenesis creates VFAs, which are acidifying compounds. When the VFAs are consumed by methanogenesis or acetogenesis, the acidifying effect is removed. However, manure has a large buffering capacity which keeps the pH relatively stable (Lyberatos and Skiadas 1999; Mao et al. 2015). The buffering capacity of manure is often attributed to dissolved ammonia and sometimes to bicarbonate (Lyberatos and Skiadas 1999). Lastly, hydrolysis also has an optimal pH range and has been observed to be inhibited at low pH (<5.6) (Alexander 1977; Mao et al. 2015). Inhibition of hydrolysis can prevent methanogen substrates from being created, and also inhibits ammonia mineralization.

2.2.6. Inoculum

Inoculum in animal manure systems is residual manure with an established or acclimated microbial population. The presence of an inoculant is hard to prevent in on-farm manure storages, as there is little value for farmers to fully clean something that will continually receive animal waste. Remaining manure in emptied storages therefore acts as an inoculant for new manure (Jayasundara et al. 2016). Current research shows that an inoculum will decrease the methanogen lag phase and increase overall emissions (Jayasundara et al. 2016). Although there

seems to be a trend of increasing CH_4 production with increasing inoculum quantity, the results are still inconclusive, especially in quantifying how much old manure needs to be removed to reduce the inoculating effect (Massé et al. 2008; Jayasundara et al. 2016; Ngwabie et al. 2016). For example, Wood et al (2014) found a 56% reduction in total GHG emissions when tanks were emptied completely compared to partially. Additionally, it is unclear how effective left-over manure is at being an inoculant and how the manure age and composition change its inoculant capacity. For example, Baldé et al (2016) emptied a manure tank in the fall and expected that with the low winter temperatures the remaining sludge would be a poor inoculant. However, the following spring/summer they observed the highest CH_4 emissions of their 3-year study period (Baldé et al. 2016b). Sommer et al (2007) noted that as little as 8% inoculant can notably reduce the lag phase in manure storage and Ngwabie et al (2016) found a 36% increase in overall emission when a tank had only 5% inoculum present compared to none (Sommer et al. 2007; Jayasundara et al. 2016; Ngwabie et al. 2016). More research is needed to fully understand when and why old manure acts as an inoculant and how quantity, age, and composition of the manure affect its viability as an inoculant.

2.3. Nitrification/Denitrification

The nitrogen cycle is a complex system with N moving between organic and inorganic forms (Figure 2.4). New concepts in N cycling provide greater complexity in the processes occurring, such as anaerobic ammonium oxidation (anammox), single reactor system for high activity ammonium removal over nitrite, (SHARON-anammox), completely autotrophic nitrogen removal over nitrite (anammox-CANON) (Kindaichi et al. 2016). This dissertation, however, focuses more on CH_4 production, which is the predominant GHG and will not discuss these advanced processes and their relation to N_2O production from manure. Future work should explore N cycling processes in storage dairy manure in more detail.

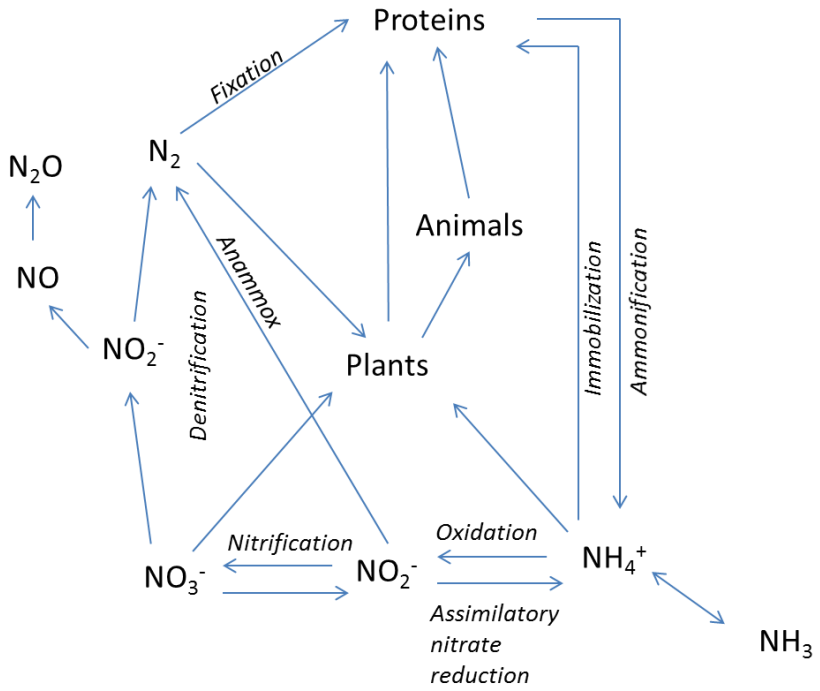

Figure 2.4 The main reactions of nitrogen cycling (Bothe et al. 2007)

Ammonium is an important form of nitrogen in liquid dairy manure, which is present in large quantities due to excretion of nitrogen in urine (Petersen et al. 2016). Ammonium (NH_4^+) is dissolved in manure, however it is easily converted to ammonia (NH_3) and lost due to volatilization. Ammonia gas is dangerous to human health, the environment, and an indirect source of N_2O (VanderZaag et al. 2009; IPCC 2014; Petersen et al. 2016). It can bind to particulate matter in air or react with secondary inorganic aerosols, creating particulate matter ($PM_{2.5}$), which can cause respiratory problems in humans (Petersen et al. 2012; Wu et al. 2016). Once the particulate matter settles in the environment it causes acidification of water and soils, and can result in N_2O production (Petersen et al. 2012; IPCC 2014). Additionally, the loss of nitrogen through NH_3 emissions represents a loss of nutrients from the manure (VanderZaag et al. 2009; Petersen et al. 2016).

Ammonia volatilization is affected by environmental factors, such as motion from wind or rain, pH, or temperature (VanderZaag et al. 2010a). If ammonium stays dissolved in manure it can eventually undergo nitrification if presented with an aerobic environment. Liquid manure storages are anaerobic, however, surface crusting creates an environment where substrates are exposed to oxygen from air and nitrification can occur. Feedlots, solid/liquid separation, dry manure piles, and field application are all cases of where nitrification can occur due to aerobic conditions. Nitrification occurs between 5 – 40℃, but the optimal temperature is 30-35℃ (Alexander 1977). Nitrification is disrupted at pH <6 and in general it is sensitive to environmental change. The main product of nitrification is nitrate (NO_3), although N_2O has been observed as a by-product in oxygen limiting environments (Figure 2.5) (Firestone and Davidson 1989). High N_2O emissions, however, are usually associated with denitrification (Firestone and Davidson 1989).

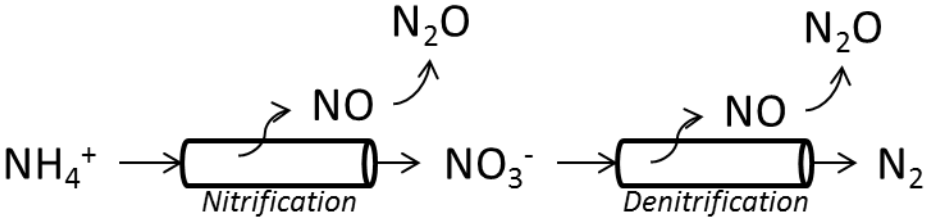

Figure 2.5 Conceptual model of N_2O loss from nitrification and denitrification (Modified from Firestone and Davidson, 1989)

Denitrification results in gaseous loss through NO and N_2O products of microbial reduction of NO_3^- (Alexander 1977). It is an anaerobic process with nitrogen gas (N_2) as its final product (Firestone and Davidson 1989). N_2O is produced as a by-product when nitrate and carbohydrates are readily available (Figure 2.5) (Alexander 1977). This is the case in manure, where NO_3^- is often limiting, therefore when it is produced aerobically in microsites, the surrounding anoxic environment readily reduce it further. When nitrate is more abundant then carbohydrates, N_2O

production can be further increased (Firestone and Davidson 1989). However, when N_2O is trapped close to the manure surface, the microbial communities have the potential to further reduce N_2O to N_2 (Alexander 1977).

Denitrification productivity decreases below pH 7, with optimal pH around 8 (Alexander 1977). And it has been observed to occur between 2-50℃, however there is a big drop in productivity below 10℃ (Alexander 1977; Firestone and Davidson 1989). Lastly, increasing the O_2 levels in the environment have been observed to reduce denitrification (Alexander 1977).

2.4. Process trade-offs

Before we address mitigation strategies fully, it is important to look at how the GHG processes work together. Although they are presented as linear, unidirectional processes, in reality all of these processes will be occurring simultaneously within manure, often competing for substrates. It is important to keep these processes in context of a whole-farm system, as there are many possible points of nutrient loss, but the end goal is to conserve nutrients for plant uptake. Table 2.1 shows the main processes discussed along with the optimal environmental and substrate conditions.

Table 2.1 Preferred environmental conditions for biochemical degradation processes (Alexander, 1977; Lyberatos and Skiadas, 1999; Yu and Fang, 2003; Atia and Government of Alberta, 2008; Vavilin et al., 2008; Mao et al., 2015).

	Temperature (℃)	pH	Oxygen	C:N
Hydrolysis	40-60	<5.6	low	—
Acidogenesis	>20	5.5-6.5	—	—
Methanogenesis	5-70	6.5-8.2	absence	high
Ammonia volatilization	>25	>7.5	—	low
Nitrification	5-40	>6	presence	—
Denitrification N_2O	2-50	>7	absence	low

The substrate availability in manure is quite large, with little need for processes to compete, however, some are sensitive to a specific C:N ratio. Methanogenesis is inhibited when the C:N

ratio is too low, due to ammonia inhibition, while during denitrification, a low C:N will cause increased N_2O production. In terms of mitigation, increasing the VS in manure could reduce CH_4 production, however if the addition also creates a crust, it could promote nitrification and N_2O emissions.

Oxygen presence is another important factor. Methanogenesis and denitrification are anaerobic processes, while nitrification requires oxygen. Although increasing O_2 by agitation, solid/liquid separation, or manure drying might reduce CH_4 production, it would also consequently increase nitrification. Unlike methanogenesis, denitrification does not require strict anaerobic conditions and can occur in anaerobic microsites. Additionally, agitation of manure would cause volatilization of ammonia. Measuring the redox potential of the manure can tell us if the reactions occurring are aerobic or anaerobic. The redox potential expresses the reduction potential of a species, or rather, its ability to accept electrons (Sommer et al. 2013). Aerobic processes use O_2 as electron acceptor and are associated with a redox value of >13 pE, while anaerobic processes can use other substrates as electron acceptors and are associated with a redox value of <-3 pE (Sommer et al. 2013).

Although there is overlap between processes, each has a specific pH range. If pH increases too much, then acidogenesis would likely stop. This means that CH_4 production would only occur until substrates present are already used, however, no new VFAs would be formed. This would potentially decrease the total CH_4 production from the manure. However, an increased pH could also increase ammonia volatilization. On the other hand, reducing pH would reduce CH_4 production and ammonia volatilization, but also increase hydrolysis and acidogenesis. This would increase the methanogenic substrates and dissolved ammonia in the manure. Although this

is beneficial, pH increases over time might increase ammonia volatilization and nitrification during field application.

The temperature ranges for these processes are still quite similar. Below ~10℃ most processes will stop or slow drastically. This means that cold weather storage is preferable when mitigating emissions. However, only winter storage is not possible as animals create manure year-round.

2.5. Manure Storage

In order to reduce GHG emissions from manures storage, GHGs can be trapped and converted into other forms, or they can be prevented by making the environment inhospitable for the microbial activity.

2.5.1. Trapping Biogas

Trapping biogas can be done with covers or specialised equipment, such as an anaerobic digester. Covers can be from natural crusting or synthetic materials. Crusts naturally form on the slurry surface as light-weight solids float to the top and congeal (Petersen et al. 2005; Owen and Silver 2015). The crust disrupts the liquid surface of the slurry and allows for a mixed environment that is both anaerobic and aerobic (Owen and Silver 2015). This allows for nitrification of N_2O and the oxidation of CH_4 to occur (Owen and Silver 2015). It creates a trade-off of decreasing CH_4 and increasing N_2O. Synthetic covers, on the other hand, physically block or slow gas movement forcing CH_4 oxidation and N_2O reduction to N_2 (Alexander 1977; Owen and Silver 2015). Gas that is trapped by a cover or other system can be additionally treated through biofiltration, or flaring (Janzen 2008). Lastly, anaerobic digestion is a way of utilizing biogas for electricity production, and is considered a preferred way of managing manure.

2.5.2. Environmental Control

Changing the environment to reduce emissions can be tricky, due to trade-offs discussed in the previous section. A common manure management practice is solid-liquid separation, where the

solids containing methanogen substrates are removed from the liquid (Møller et al. 2004a). Some technologies used to remove high substrate solids include: screwpress, decanting centrifuge, and chemical treatments (Møller et al. 2004a; VanderZaag et al. 2017). Although high CH_4 production can be achieved in the liquid portion, and unless the solids are well dried there is possibility of increased N_2O production (Alexander 1977).

Acidification has shown good results in GHG reductions (Kai et al. 2008). NH_3 volatilization is reduced by shifting the equilibrium of NH_4^+ and NH_3 to ammonium (NH_4^+) and hence keeping it in solution (Clemens et al. 2002). This is especially important for swine manure, which is higher in ammonia. Depending on how low the pH becomes, it can disrupt methanogenesis and even acidogenesis. It might, however, increase the hydrolysis due to chemical breakdown of OM. When using acidification, timing of storage will be important as the pH naturally rises and may rapidly release dissolved ammonia once the pH is high enough.

Lastly, tank cleaning and removing inoculum is considered a best management practice (Janzen 2008). Reducing inoculum will increase the lag time for CH_4 emissions, as well as overall emissions.

3. Greenhouse Gas Emissions from Gradually-Filled Liquid Dairy Manure Storages with Different Levels of Inoculant

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3.1. Introduction

In Canada, liquid dairy manure storage is a typical practice, with usually >100 d of storage (Sheppard et al. 2011). During this time, significant greenhouse gases (GHG) such as methane (CH_4) and nitrous oxide (N_2O) are produced (VanderZaag et al. 2010b; Jayasundara et al. 2016). Additionally, ammonia (NH_3) volatilizes from manure, which leads to N deposition in sensitive ecosystems causing eutrophication, acidification of water systems, and may be re-emitted as N_2O following deposition (Krupa 2003; Kavanagh et al. 2019). Mitigating these emissions is important for both farmers and the environment, as loss of nutrients from manure depreciates its fertilizer value and GHGs contribute (CH_4 and N_2O) towards climate change. Better understanding of gaseous emissions from agricultural systems is important for finding reduction strategies and assessing predictive modelling tools.

On dairy farms, manure is constantly produced and gradually loaded into storage tanks before being applied to fields. Most experimental GHG emissions research, however, has utilized batch filling into meso-scale tanks or incubated jars (Massé et al. 2003; Møller et al. 2004b; VanderZaag et al. 2010a, 2017; Wood et al. 2014a; Ngwabie et al. 2016; Le Riche et al. 2017; Baral et al. 2018). In anaerobic digestion, the substrate to microorganism ratio is key to controlling digestion, as lower ratio means more of the substrate will be digested and converted

to gas (Burke 2001). Additionally, high levels of substrate can unbalance the microbial reactions, and cause an accumulation of acidic compounds which are toxic to methanogens (Lyberatos and Skiadas 1999). As such, in the context of on-farm manure storage, gradual filling of smaller amounts of manure might enhance microbial activity and emissions due to the addition of fresh substrates and reduced chances of toxicity. This suggests that current research may be underestimating GHG emissions when utilizing batch filling. Understanding the effect that filling has on GHG production is important for building more accurate predictive models. Currently there has been no research comparing gradual and batch filling of liquid manure on subsequent GHG emissions.

Within manure storages the microbial activity is largely affected by the presence of inoculating cultures. Old manure remaining in storages is known to act as an inoculum and subsequently increases GHG emissions (Sommer et al. 2007; Jayasundara et al. 2016; Ngwabie et al. 2016; Habtewold et al. 2018a). Sommer et al. (2007) noted that as little as 8% inoculant can reduce the lag phase (initial period of low emissions) in manure storage. Wood et al. (2014) found that complete removal of inoculum resulted in a 50% reduction of GHG emissions when the tanks were re-filled. Ngwabie et al. (2016) found a positive linear relationship of cumulative CH_4 emissions and inoculum levels (0, 5, 10, 15, 20 and 25%).

Given that both the inoculum level and the filling type both have the potential to influence GHG emissions, there is a need to evaluate the compounding effect of these on emissions from stored liquid manure. This study assessed the effect of gradual and batch filling of tanks and different inoculum levels (0%, 10%, and 20%) on the production of CH_4, N_2O, and NH_3, and the total CO_2-equivalent GHG emissions over 122 d of warm-season storage.

3.2. Methods

3.2.1. Site description and tank filling

Dairy manure was stored in 6 pilot-scale, in-ground, concrete manure tanks (1.7 m deep, 6.6 m^2) at Dalhousie University's Bio-Environmental Engineering Centre in Bible Hill, NS, Canada (45°45' N, 62°50' W). Each tank was enclosed by a continuously flow-through steady-state chamber for monitoring emissions. The site has previously been described by VanderZaag et al, 2010a. This site provides a unique scale of research, because each tank contains ~11 m^3 of manure, which is more realistic than laboratory bottles (< 1 L manure), while enabling treatments to be compared under the same conditions (unlike farm manure tanks). At the same time, the research site enables high temporal resolution. On the other hand, the cost of the site operation limits the number of tanks to six. Due to these constraints, there were no treatment replications, instead this preliminary study focused on continuous flux measurement for a full season of storage which will inform future research.

Three tanks were chosen at random to be batch-filled with 11.4 m^3 of manure on 1 d (Jun 2, 2016). Three other tanks were gradually-filled, receiving 1/3 of the volume on three dates: 1 d (Jun 2), 20 d (Jun 22), and 43 d (Jul 15, 2016).

Each tank within the batch- and gradually-filled tanks was randomly assigned different inoculant level (0%, 10%, or 20%). Inoculant was prepared on May 24, by removing old manure (previously stored for about 6 months) from the tanks, mixing it and redistributing it into four cleaned tanks. The 10% inoculum tanks received 1.1 m^3 of inoculant, while the 20% inoculum tanks received 2.3 m^3 of inoculant (Table 3.1). All tanks were continuously monitored from Jun 1 to Oct 1, 2016 (122 d).

This study was performed in parallel with Habtewold et al. (2018), which focused on linking the CH_4 emissions to microbial activity within the manure.

Table 3.1 The volume (m^3) of inoculum and fresh manure in all gradual and batch fill treatments including the percentage filled (%) in parenthesis.

		Manure volume, m^3 (% full)			
		Inoculum	Day 1	Day 20	Day 43
Gradual 0% inoculum	Tank 6	0.0 (0%)	3.8 (34%)	7.6 (66%)	11.4 (100%)
Gradual 10% inoculum	Tank 1	1.1 (10%)	4.5 (40%)	8.0 (70%)	11.4 (100%)
Gradual 20% inoculum	Tank 3	2.3 (20%)	5.3 (47%)	8.3 (73%)	11.4 (100%)
Batch 0% inoculum	Tank 4	0.0 (0%)	11.4 (100%)	11.4 (100%)	11.4 (100%)
Batch 10% inoculum	Tank 5	1.1 (10%)	11.4 (100%)	11.4 (100%)	11.4 (100%)
Batch 20% inoculum	Tank 2	2.3 (20%)	11.4 (100%)	11.4 (100%)	11.4 (100%)

3.2.2. Chamber and tank set-up

To measure emissions, each tank was enclosed by a flow-through, steady-state chamber made of an aluminium frame covered by 6 mil greenhouse plastic (Livingston and Hutchinson 1995; Le Riche et al. 2017). Air was drawn into each chamber through three vents and exited through an exhaust venturi on the opposing side of the tank vents. Inflow air was sampled at two locations 1.7 m above ground on the inflow side of the tanks. Cup anemometers (7911, Davis Instruments, Hayward, CA) measured airspeed in the outflow venturi of each chamber and copper-constantan thermocouples (Omega Engineering Inc., Laval, QC) measured the air temperature 30 cm above the manure surface and manure temperature at 80 (mid-depth) and 150 cm (bottom) depth. All outputs were averaged by minute. The airspeed and surface temperature values were recorded by CR1000 datalogger (Campbell Scientific, Logan, UT) and the manure temperatures were recorded by a CR23X datalogger (Campbell Scientific, Logan, UT). Due to instrument failure there were a number of gaps in the manure temperature data at 150 cm depth. Temperature at 80 cm was near mid-depth of manure, and was considered to represent the manure temperature in

each tank for comparison purposes. Due to depth changes the temperature in the gradually-filled tanks is not reported until all tanks reached the same manure volume on Jul 15 (43 d).

3.2.3. Methane and Nitrous Oxide

Air samples were automatically drawn (RC0021, Busch Vacuum Pumps and Systems, Boisbriand, QC, CA) from each sampling location (6 tanks and 2 ambient inflow location), through polyethylene tubing (3.2 mm i.d.; Rubberline Products Ltd., Kitchener, ON) into a 8×2 manifold (Campbell Scientific In., Logan, UT) containing 12 V DC valves (The Lee Co., Essex, CT). The valves were programmed to select two different air sample locations every 30 sec whose air flow was directed into high-flow air dryers (Perma Pure LLC.; Toms River, NJ) before entering one of the two tunable diode laser trace gas analyzers (TDLTGA, Campbell Scientific, Logan, UT). A CR5000 datalogger (Campbell Scientific Inc., Logan, UT) recorded the data from each analyzer and a PC computer was continuously running with TDLTGA software to monitor the analyzer and download data from the CR5000.

The gas fluxes from each manure tank were calculated according to this equation (Livingston and Hutchinson 1995; Le Riche et al. 2017):

$$\boldsymbol{F = \frac{Q}{A}(C_o - C_i)} \qquad \text{Eq 3.1}$$

where F is the flux (e.g. mg m^{-2} s^{-1}), Q is the flowrate of air out of the chamber (air speed measured in the venturi × cross-sectional area of venturi (0.0645 m^2), m^3 s^{-1}), A is the surface area of the manure tank (6.63 m^2), and C (mg m^{-3}) is the concentration of gas in the inlet air (C_i) and outlet air (C_o). Due to technical issues, linear interpolation was used to fill CH_4 and N_2O flux data gaps on dates Jun 29 to Jul 7, 2016 and Sep 15 to 19, 2016.

3.2.4. Ammonia

Ammonia gas was captured using 0.005 M H_3PO_4 acid traps. Air samples were pulled through 25 m of polyethylene tubing at a rate of 1.5 L min^{-1} using a vacuum pump (Model 2107CA20B; Thomas Pumps and Compressors, Sheboygan, WI) and bubbled through 125 ml of acid using dispersion tubes (id = 35 mm). Air flow for each sample was measured using inline flow meters (Gallus 2000; Actaris Metering Systems, Greenwood, SC). The system was deployed for 24 h 3× per week. During liquid collection, additional acid solution was added to standardize the volume to 125 ml to correct for evaporation. The liquid was analyzed for NH_3-N at Agriculture and Agri-Food Canada (Ottawa, ON) using the QuikChem® Method 12-107-06-2-A modified for 0.005 mol L^{-1} H_3PO_4 matrix using a Lachat QuikChem FIA+ Q8500 Series (Hofer 2003). The gas concentrations were calculated as:

$$C_{NH_3\ air} = \frac{C_{NH3\ aq} \times V_{aq}}{V_{air}} \qquad \text{Eq. 3.2}$$

where $C_{NH_3\ air}$ is the NH_3-N concentration in gas (mg m^{-3}), $C_{NH3\ aq}$ is the NH_3-N concentration in liquid (mg m^{-3}), V_{aq} is the volume in the acid trap (m^3), and V_{air} is the volume of air pumped through the acid (m^3) (Hofer 2003).

3.2.5. Manure

Liquid dairy manure was obtained from a near-by dairy farm with ~100 lactating cows that used washed quarry sand for bedding (the same farm manure was used in LeRiche et al. 2016: "M1"; and LeRiche et al. 2017). Manure was obtained when fresh manure was being pumped into the outdoor storage.

Manure samples from the experimental tanks were taken monthly as a composite of 12 locations in each tank (6 locations in a grid and 2 depths at 80 and 160 cm from the surface). Samples

were frozen until analyzed at the Nova Scotia Department of Agriculture's Provincial Soils Lab in Bible Hill, NS. Samples were analyzed for dry matter (DM) and volatile solids (VS) according to American Public Health Association (APHA) method 2540 B, total nitrogen (TN) according to combustion method (AOAC 990.03-2002), ammonium-N (TAN) according to APHA 4500-NH_3 B, and pH using an electrode according to APHA 4500-H^+ (Clesceri et al. 1998). On Aug 5, Sep 24, and Oct 16, additional samples were collected for analysis of volatile fatty acids (VFAs). These samples were kept frozen until shipped to InnoTech Alberta Laboratory (Vegreville, AB). The VFA analysis was done through headspace gas chromatography using a DB-FFAP column on a Varian CP-3800 gas chromatograph with a flame ionization detector (Agilent Technologies, Santa Clara, CA) as described by Apelt et al. (2016). Individual VFA concentrations were calculated by comparing peak areas corresponding to calibrated standards of formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, 4-methylvaleric acid, hexanoic acid, and heptanoic acid (Apelt et al. 2016).

Supplemental water was applied on a weekly basis to offset volume loss due to evaporation. Each tank, therefore received a unique volume of water based on evaporative loss. Water was added using a sprinkler to simulate rainfall and measured each watering day using a flow meter.

3.2.6. Data Analysis

For a more direct comparison, cumulative gas fluxes were also scaled by the volume of manure as:

$$\boldsymbol{F_v = \frac{F}{h}} \qquad \text{Eq. 3.3}$$

where F_v is the flux scaled by volume (g m^{-3}), F is the flux scaled by area (g m^{-2}), h is the depth of the manure within the tank. Tank volumes are shown in Table 3.1.

To account for variation between tanks of available N in the fresh manure, the cumulative N_2O and NH_3 flux was scaled by total TN and TAN (kg $tank^{-1}$) in fresh manure. Similarly, the daily CH_4 flux was scaled by total VS (kg $tank^{-1}$) in fresh manure, which represents the available substrates for methane production. Additionally, the methane conversion factor (MCF), which is the ratio of CH_4 produced compared to the maximum potential CH_4 (B_o), was calculated following methods published by the Intergovernmental Panel on Climate Change (IPCC) (Dong et al. 2006). The B_o used to calculate the MCF was the IPCC default value of 0.24 m^3 CH_4 kg^{-1} VS (Dong et al. 2006).

Total GHG emissions for each tank were calculated as a sum of CH_4, direct N_2O, and indirect N_2O on a 100-yr CO_2-eq basis to compare on the basis of their global warming potential. The global warming potentials used for conversions were 34 for CH_4 and 298 for N_2O (IPCC 2014). The conversion factor for calculating indirect N_2O-N from NH_3-N was 0.01 (Dong et al. 2006).

For all averaged data, the standard error of the mean (SEM) was calculated and expressed as mean±SEM in tables or text and as error bars in figures.

3.3. Results

3.3.1. Environmental and Manure Parameters

The average ambient air temperature at the nearest Environment Canada weather station (Debert, NS, Station ID 8201380, ~15 km from the research site) from Jun to Oct was 15°C. The monthly averages were within 1°C of the 30 y normals. For Jul and Aug, when the CH_4 emissions and ambient air temperature were the highest, the average manure temperature in the gradually-filled tanks (19.6±0.081°C) was 2.2°C warmer than in the batch-filled tanks (17.4±0.074°C). Over the monitoring period (final filling date Jul 15 to Oct 1), the gradually-filled tanks had on average 1.8°C warmer manure compared to the batch-filled tanks (19.1±0.11°C vs 17.3±0.06°C; Figure

3.1). This difference was not seen at 150 cm manure depth, however, where the gradually and batch-filled tanks were consistently similar over the entire monitoring period (14.5±0.11°C and 14.7±0.06°C, respectively).

The VS of fresh manure was on average 49±1.78% (dry basis), which decreased to 42±0.89% at the end of the trial (Sep 24) (Table 3.2). During storage, VS content dropped in all tanks except the 0% gradually-filled tank (Tank 6) (Table 3.2). The DM of the fresh manure was on average 14.7±1.36% (max = 19.9%; min = 10.7%; Table 3.2). The DM, including sand from the bedding, settled into a thick sludge layer deposited at the bottom of the tanks (~0.5 m thick).

The average N of fresh manure was 2.5±0.14% (dry basis), while TAN was 0.6±0.042% (Table 3.2). The average pH of the fresh manure was 7.0±0.03 but increased to pH 7.8±0.03 at the end of the study (Sep 24). There were no marked differences in N, TAN, or pH between tanks (Table 3.2).

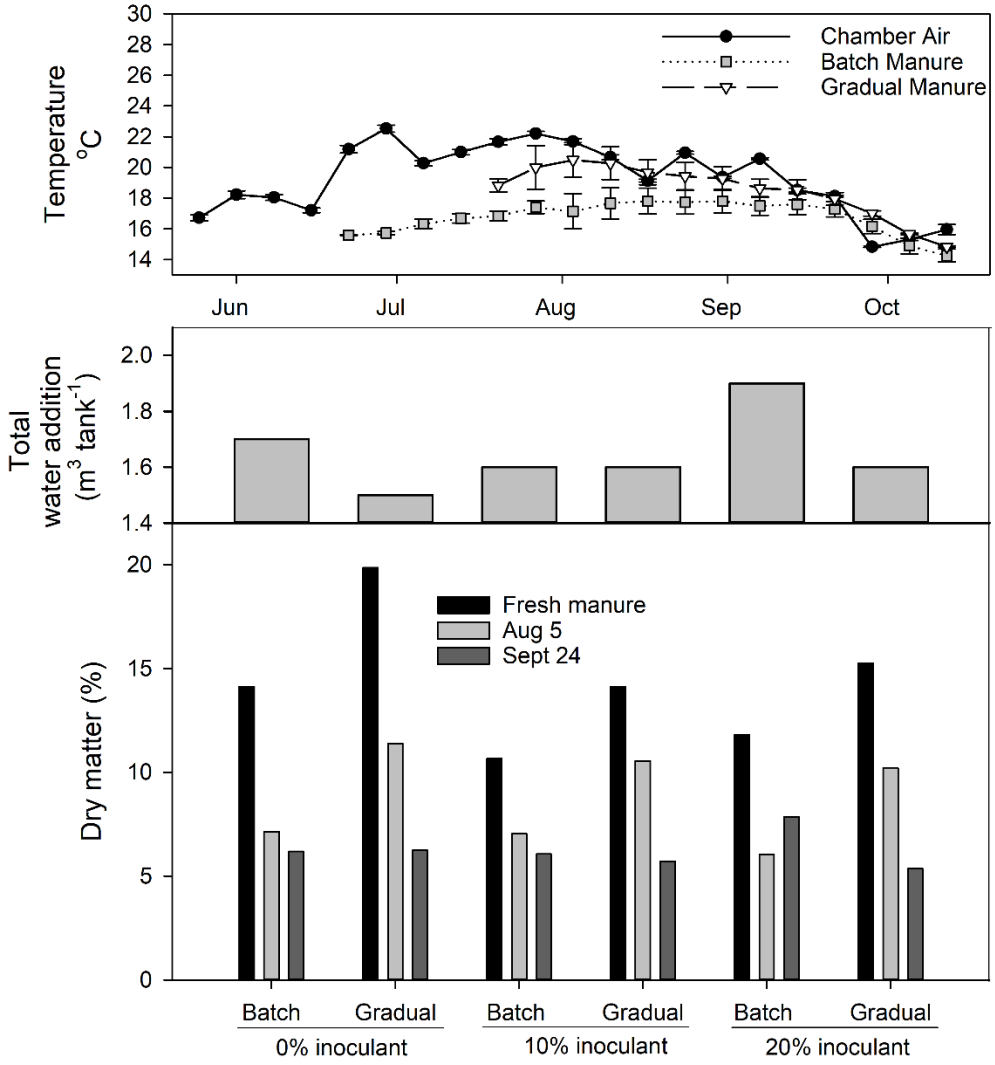

Figure 3.1 Average chamber air and 80 cm depth manure temperature (°C) (figure top), total water addition (m^3) to each tank (figure middle), and manure dry matter (%) from start (2-Jun), middle (5-Aug) and end (24-Sep) of study (figure bottom). Error bars represent standard error of the mean (N=3).

Table 3.2 Manure and inoculant characteristics including dry matter (DM), volatile solids (VS), pH, nitrogen (N) and ammonium-N from three different dates (Fresh manure on Jun 2, 5-Aug, and 24-Sep, 2016).

		0% inoculant		10% inoculant		20% inoculant	
		Batch	Continuous	Batch	Continuous	Batch	Continuous
		Tank 4	Tank 6	Tank 5	Tank 1	Tank 2	Tank 3
DM (%)	Inoculant	-	-	2.3	2.3	2.3	2.3
	Fresh	14.1	19.9	10.7	16.5	11.8	15.3
	5-Aug	7.1	11.4	7.0	10.6	6.1	10.2
	24-Sep	6.2	6.3	6.1	5.71	7.9	5.4
VS (%) dry basis	Inoculant	-	-	52.9	52.9	52.9	52.9
	Fresh	47.6	42.0	53.0	46.6	53.8	48.7
	5-Aug	49.1	43.4	50.8	41.1	50.3	40.9
	24-Sep	42.0	42.4	41.1	39.8	45.2	39.0
pH	Inoculant	-	-	7.6	7.6	7.6	7.6
	Fresh	6.9	7.0	7.1	7.0	7.1	7.0
	5-Aug	7.6	7.6	8.0	8.0	7.9	7.8
	24-Sep	7.7	8.2	7.7	7.8	7.8	7.6
N (%) dry basis	Inoculant	-	-	4.5	4.5	4.5	4.5
	Fresh	2.7	2.0	2.8	2.2	2.8	2.3
	5-Aug	3.2	2.2	3.5	2.2	2.6	1.9
	24-Sep	4.0	4.0	3.3	3.5	3.4	3.7
Ammonium-N (%) dry basis	Inoculant	-	-	2.2	2.2	2.2	2.2
	Fresh	0.6	0.4	0.5	0.5	0.7	0.5
	5-Aug	0.8	0.4	0.9	0.4	0.4	0.3
	24-Sep	1.7	1.8	1.0	1.1	1.2	1.1

Between the two sampling dates, Aug 5 to Sep 24, there was an 88% drop in the total VFAs in all tanks (Figure 3.2). On both dates, VFAs were highest in the 0% inoculum tanks, while the 10% inoculum tanks were 61% (Aug 5) and 97% (Sep 24) lower, and the 20% inoculum tanks were 85% (Sep 24) and 97% (Sep 24) lower (Figure 3.2). On Aug 5, all tanks had elevated propionic acid relative to other VFAs, but by Sep 24 propionic acid was only elevated in the 0% inoculum tanks.

3.3.2. Methane emissions

The average daily CH_4 emissions from all tanks were 79.5±6.60 g m^{-2} d^{-1} for the entire 122 d monitoring period and the cumulative emissions were 5.88±0.536 kg m^{-3} (Table 3.3). The CH_4

emission curves of all tanks demonstrated a lag phase as microbes established in the manure, followed by a period of rapidly increasing emissions and a subsequent decrease as temperatures declined (Zeeman 1994; Le Riche et al. 2017; Habtewold et al. 2018a). Most tanks had a similar length lag phase of ~30 d (Figure 3.3), with the exception of 0% inoculum gradually-filled tank, where the lag phase was nearly twice as long (~60 d). Once the flux peaked, there was a period of elevated emissions lasting up to 5 weeks in Jul and Aug. The variability between tanks was higher during this phase (107±17.0 g m^{-2} d^{-1}), compared to the lag phase (17.3±1.82 g m^{-2} d^{-1}) and the post-peak phase (78.2±3.55 g m^{-2} d^{-1}) (Figure 3.3). Additionally, of the total emissions from all the tanks, Jul made up 29% and Aug 44%, suggesting that differences in filling strategies were most important during the months of Jul and Aug when emissions were highest.

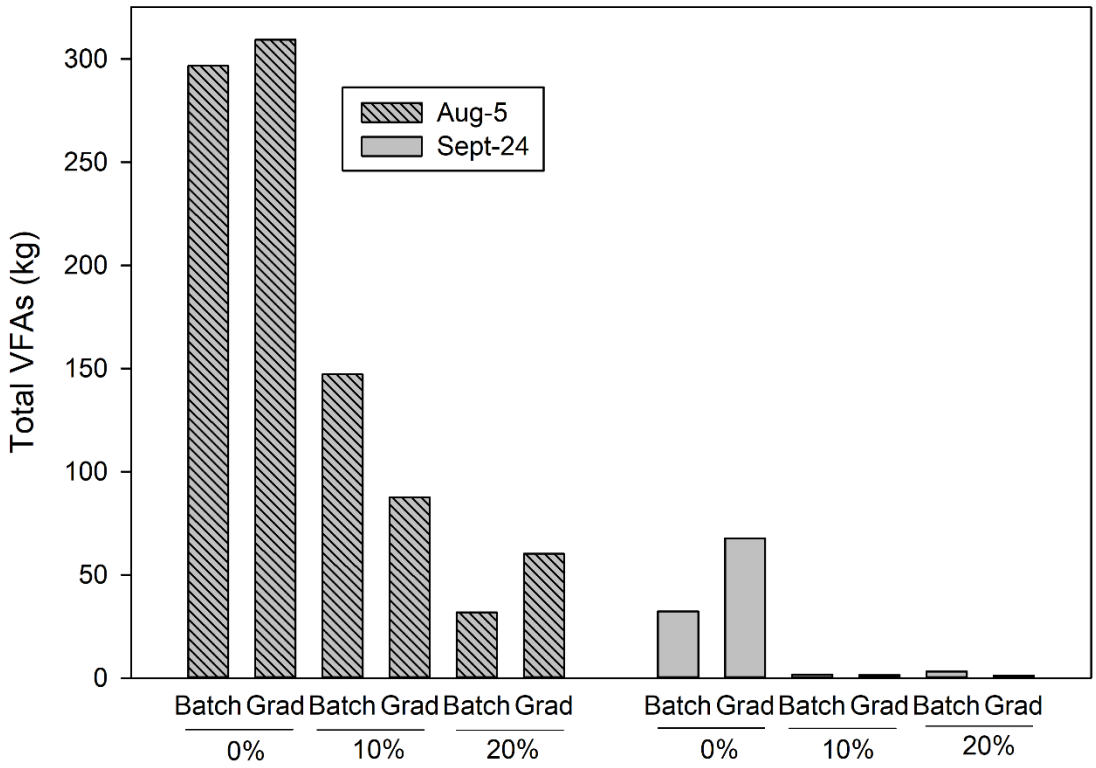

Figure 3.2 Total volatile fatty acids (VFAs; kg) in each tank at two dates (5-Aug, 2016, 64 d and 24-Sep, 2016, 114 d).

There was a small difference between the filling types, with batch-filled tanks producing 12% less CH_4 than gradually-filled tanks when scaled by manure volume (5.49±0.104 vs 6.26±1.13 kg m^{-3}). This difference was also present when scaled by VS (83.8±2.74 vs 75.4±16.2 g kg^{-1} VS) (Table 3.3, Figure 3.3).

Table 3.3 Daily emissions of methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3-N) averaged over the 122 day study. Cumulative emissions were scaled by volume of manure and surface area. Cumulative CH_4 emissions and volatile solids were used to calculate a methane conversion factor (MCF) for each tank. Cumulative N_2O and NH_3 emissions were scaled by total nitrogen (TN, g kg TN^{-1}) and total ammoniacal-nitrogen (TAN, g kg TAN^{-1}).

	0% inoculant		**10% inoculant**		**20% inoculant**		**LeRiche et al (2017)**
CH_4	**Batch**	**Gradual**	**Batch**	**Gradual**	**Batch**	**Gradual**	**207 d**
Daily mean g m^{-2} d^{-1}	79.3±4.56	53.0±4.13	74.4±5.17	99.1±6.66	77.8±4.69	93.4±6.72	16
g m^{-2}	9681	6470	9077	12095	9491	11394	3271
g m^{-3}	5648	4026	5296	7666	5537	7091	1817
g kg^{-1} VS	79.7	43.0	89.0	90.8	82.7	92.5	
MCF	0.51	0.27	0.57	0.58	0.53	0.59	0.49
N_2O							
Daily mean mg m^{-2} d^{-1}	17.0±1.56	27.5±2.21	43.8±4.11	44.3±4.35	29.3±2.43	20.9±2.60	10
g m^{-2}	2.05	3.14	5.29	5.36	3.55	2.53	1.4
g m^{-3}	1.13	1.80	2.65	2.60	1.99	1.13	0.8
g kg^{-1} TAN	1.26	1.99	5.76	3.64	2.17	1.94	1.01
g kg^{-1} TN	0.30	0.43	0.99	0.85	0.59	0.43	0.57
NH_3-N							
Daily mean g m^{-2} d^{-1}	3.06±0.11	3.79±0.16	4.67±0.21	3.85±0.15	3.20±0.15	3.85±0.15	1.9
g m^{-2}	377	467	575	473	381	473	387
g m^{-3}	220	342	335	326	222	317	215
g kg^{-1} TAN	233	299	639	329	235	376	271
g kg^{-1} TN	55.1	64.8	110	76.6	64.2	83	160

Emissions of CH_4 differed between the tanks with no inoculum (0%) and those with inoculum (10% and 20%). When CH_4 emissions were averaged by inoculum level, the 0% inoculum tanks

had the least emissions (4.84±0.811 kg m^{-3}), producing 25% less than the 10% inoculum tanks (6.48±1.19 kg m^{-3}) and 23% less than the 20% inoculum tanks (6.31±0.777 kg m^{-3}).

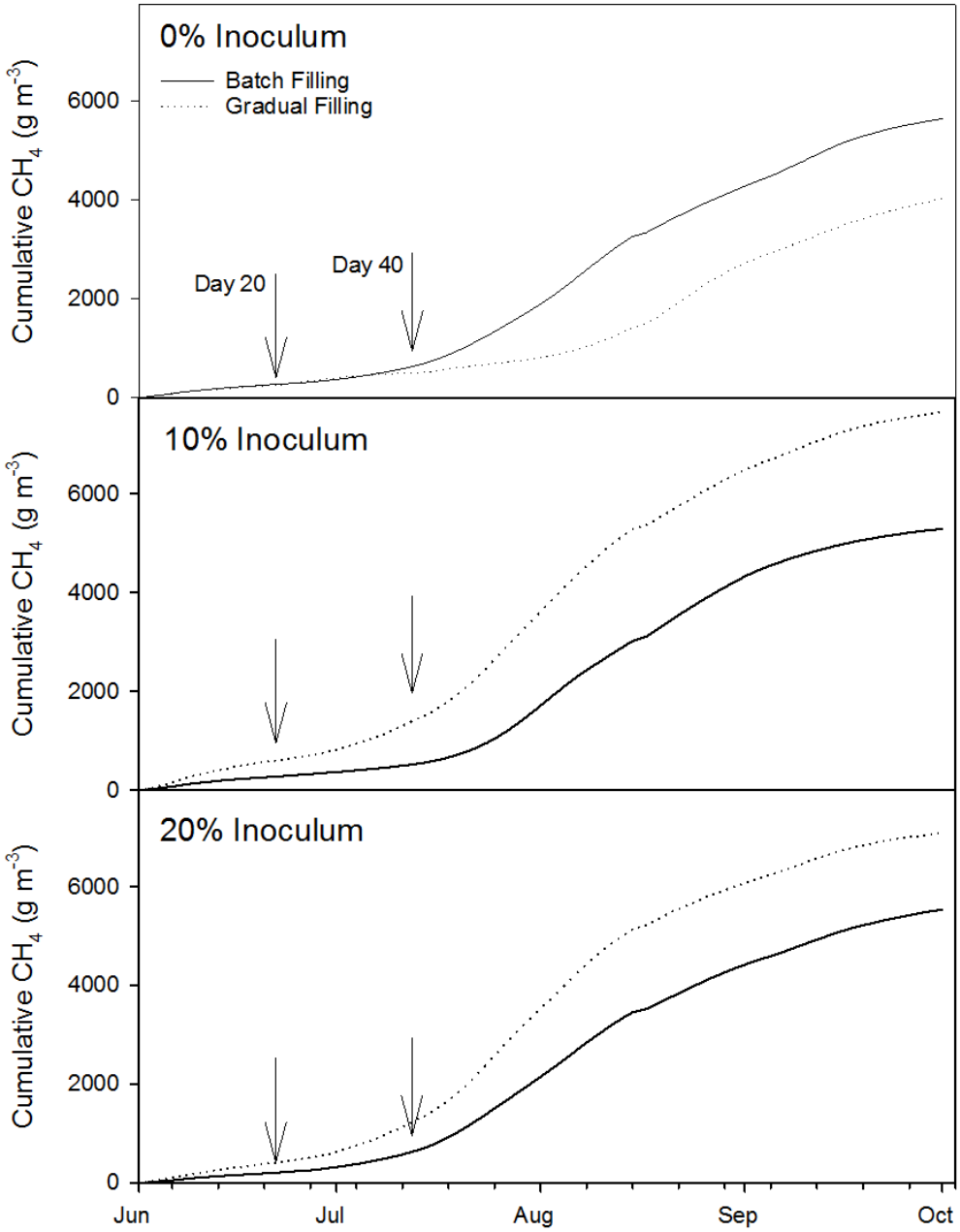

Figure 3.3 Cumulative methane (CH_4) emissions (g m^{-3}) from each tank over the entire storage monitoring period (Jun 2 to Oct 1, 2016; 122 d). Arrows indicate filling dates for gradual tanks on 20 d and 43 d.

There was no substantial difference between the 10% and 20% inoculum level tanks. Tanks containing inoculum (10% and 20%) had more CH_4 emissions in gradually-filled (7.38±0.288 kg

m^{-3}), than batch-filled tanks (5.42±0.121 kg m^{-3}) representing a 27% difference. This demonstrates that tanks with inoculum have higher CH_4 emissions in continuously-filled tanks.

In the 0% inoculant tanks, CH_4 emissions had opposite results; the gradually-filled tank had 29% fewer CH_4 emissions compared to the batch-filled tank (5.65 vs 4.03 kg m^{-3}).

3.3.3. Nitrous Oxide emissions

The average N_2O emissions from all tanks was 30.5±4.67 mg m^{-2} d^{-1} for the entire monitoring period and the cumulative emissions were 1.88±0.27 g m^{-3} over 122 d (Table 3.3). There was no marked difference in the cumulative N_2O emissions between batch-filled (1.92±0.44 g m^{-3}) and gradually-filled tanks (1.84±0.43 g m^{-3}) as shown in Figure 3.4.

The amount of inoculum did not seem to have a discernible effect on the amount of N_2O emissions. The largest emissions were from the tanks with 10% inoculum (2.63±0.02 g m^{-3}), which was nearly double both the 0% inoculum (1.46±0.34 g m^{-3}) and the 20% inoculum (1.56±0.43 g m^{-3}) tank emissions. This pattern remained similar when N_2O emissions were scaled by TAN and TN (Figure 3.4).

3.3.4. Ammonia

The average NH_3 emissions from all tanks were 3.7±0.36 g m^{-2} d^{-1} and cumulative emissions were 294±24.4 g m^{-3} over the 122 d monitoring period (Table 3.3). The gradually-filled tanks produced consistently higher NH_3 emissions throughout the study (Figure 3.4). In total, they produced 28% more on a volume basis (259±38.1 vs 328±7.16 g m^{-3}). The largest difference between filling type was in June, where the batch filled tanks emitted 53% less NH_3 (42.7±5.36 vs 80.3±9.62 g m^{-3} $month^{-1}$). From Jul to Sep batch filled tanks emitted on average 24% less NH_3, with largest occurring throughout the month of Aug (102±2.98 vs 72.3±17.1 g m^{-3} month-

[1]). By the last two weeks of the monitoring period, emissions from gradual and batch-filled tanks were nearly identical (32.8±1.86 vs 31.0±0.59 g m^{-3}).

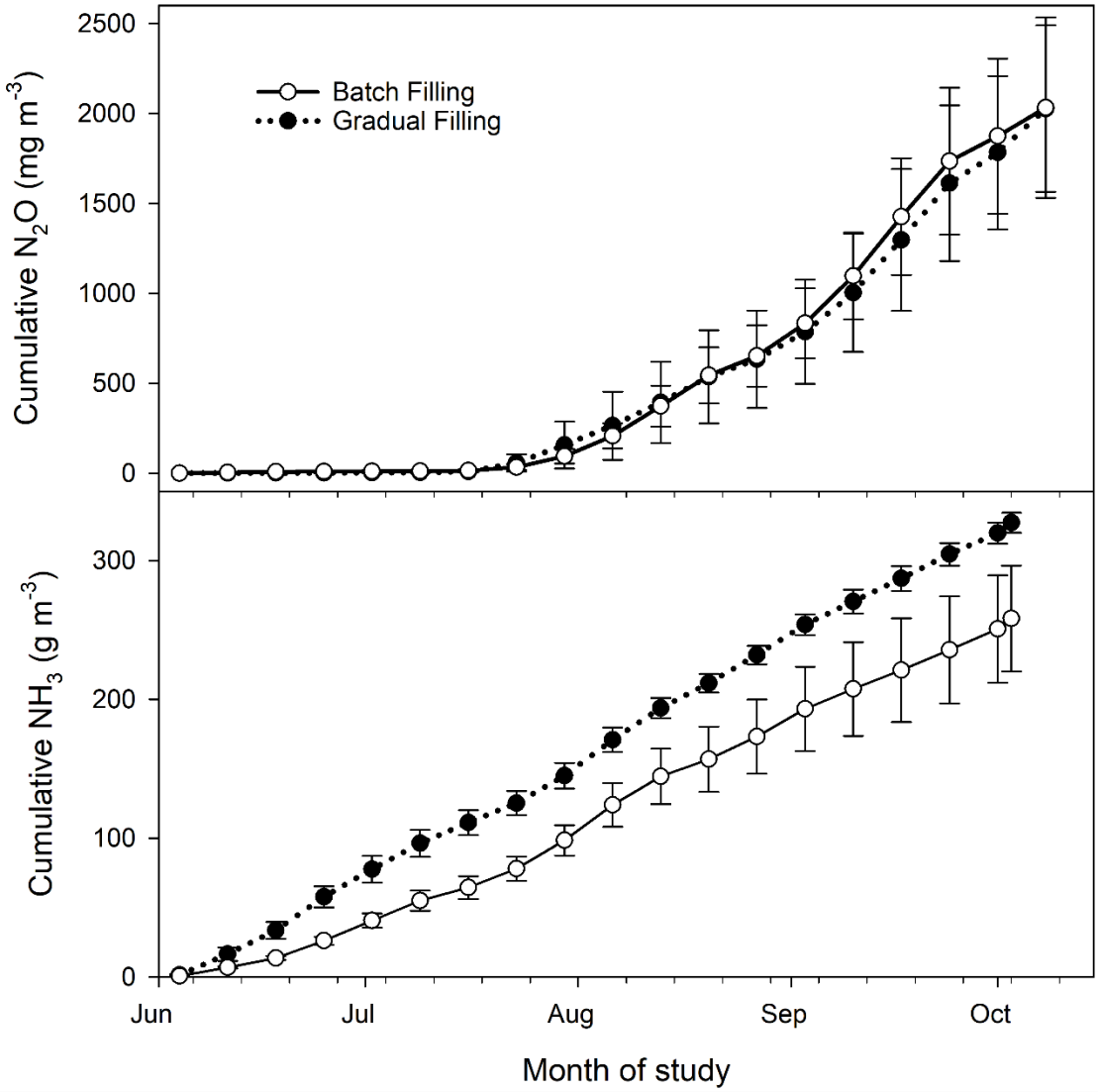

Figure 3.4 Average cumulative nitrous oxide (N_2O) and ammonia (NH_3) emissions from batch and gradual tanks for the entire study period (Jun 2 to Oct 1, 2016; 122 d). Error bars represent standard error of the mean (N=3).

The amount of inoculum did not correlate linearly with NH_3 emissions. On average, tanks with 10% inoculum emitted the most NH_3 (331±4.73 g m^{-3} or 524±50.8 g m^{-2}) and the 0% (281±60.9 g m^{-3} or 422±44.9 g m^{-2}) and 20% (270±47.4 g m^{-3} or 427±46.1 g m^{-2}) inoculum tanks differed from each other by <5%.

3.3.5. CO_2-Equivalent Emissions

For all tanks, the N_2O emissions from direct (N_2O) and indirect (NH_3-N) sources contributed < 2% of the total CO_2-eq GHG emissions (Table 3.4). The remaining 98% was from CH_4 which was due to the anaerobic environment in liquid manure which agrees with what has previously been reported (VanderZaag et al. 2009; Wood et al. 2013; Le Riche et al. 2017). Overall, gradually-filled tanks emitted 12.4% more CO_2-eq GHGs on a volume basis compared to batch-filled tanks. Considering only inoculated tanks, gradually-filled tanks emitted 26.5% more than batch-filled inoculated tanks. The 0% inoculum tanks emitted 24.2% fewer GHGs on a volumetric basis compared to the 10% and 20% inoculum tanks.

Table 3.4 Total greenhouse gas (GHG) emissions expressed as CO_2-equivalents (kg CO_2-eq m^{-3} and kg CO_2-eq m^{-2}) including methane (CH_4), direct nitrous oxide (N_2O-direct), and indirect N_2O from ammonia emissions (N_2O-indirect), from all tanks for the entire study (Jun 2 to Oct 1, 2016, 122 d).

GHG Emissions	0% inoculant		10% inoculant		20% inoculant	
kg CO_2-eq m^{-3}	Batch	Gradual	Batch	Gradual	Batch	Gradual
CH_4	192	137	180	261	188	241
N_2O - direct	0.3	0.5	0.8	0.8	0.6	0.3
N_2O - indirect	1	1.6	1.6	1.5	1	1.5
Total	**193**	**139**	**182**	**262**	**189**	**243**
kg CO_2-eq m^{-2}						
CH_4	329	220	309	411	323	387
N_2O - direct	0.6	0.9	1.5	1.6	1.0	0.7
N_2O - indirect	1.7	2.2	2.7	2.2	1.8	2.2
Total	**331**	**222**	**312**	**414**	**325**	**390**

3.4. Discussion

Our results are comparable to those by LeRiche et al (2017) who monitored manure mixed with sand bedding from the same farm for 207 d (Table 3.3). Our study produced >50% more total CH_4 (g m^{-2}) compared to LeRiche et al (2017). This was likely due to the higher VS content

(26%) of the manure in this study which was double that of LeRiche et al (2017). This is reflected by the MCF values which were slightly higher in our study (Table 3.3).

Gradually-filled tanks produced on average more (12.3%) CO_2-eq GHGs compared to batch-filled tanks due to contributions of CH_4 and NH_3, while filling type had little effect on N_2O emissions. It is important to note, that 100% of the manure in batch filled tanks was stored for 122 d, while in the gradually filled tanks 1/3 manure volume was stored for 122 d, another 1/3 volume for 102 d, and the last 1/3 volume for only 79 d. Therefore, if emissions were scaled by average storage length (101 d – gradual and 122 d – batch), the difference in emissions becomes larger (27%).

Increased emissions could be related to the higher manure temperature observed in gradually-filled tanks. Temperature is an important factor for both NH_3 volatilization and CH_4 production. NH_3 volatilization is temperature dependent, where NH_3 solubility in liquid decreases as temperature increases (Dewes 1996; Van der Stelt et al. 2007). Similarly, CH_4 production is known to increase with rising temperature (Massé et al. 2003; VanderZaag et al. 2010a). In fact, temperature differences, even at low ranges, have been shown to change CH_4 emissions markedly. For example, Massé et al. (2003, 2008) measured CH_4 emissions from various slurry types at temperatures between 10°C and 20°C and found consistently higher (50-65%) emissions at higher temperatures. The IPCC MCFs increase by 22% for a 2°C increase in temperature, i.e. 0.32 at 17°C and 0.39 at 19°C for liquid slurry (Dong et al. 2006). Therefore, the 12% increase in CH_4 emissions observed in this study is consistent with the gradually-filled tanks having 1.8°C warmer manure compared to batch-filled tanks (19.1±0.11°C vs 17.3±0.06°C).

The presence of inoculum increased overall CO_2-eq GHG emissions, due to increased emissions of CH_4. This is consistent with Wood et al. (2014), who found that tanks with inoculum had a shorter CH_4 production lag phase, which indicates higher microbial growth compared to tanks with no inoculum. Microbial growth is reflected in the VFA results, where tanks with inoculum had consistently the lowest amounts. The breakdown of organic matter in the manure creates VFAs, which are further degraded by methanogens to produce CH_4 (Lyberatos and Skiadas 1999; Mao et al. 2015). Therefore, lower VFAs reflect continued microbial activity as CH_4 is produced. Indeed, Habtewold, et al (2018) reported a higher abundance of methanogens and bacteria in tanks with inoculum compared to tanks with no inoculum.

Ngwabie et al. (2016) reported a linear relationship between inoculum level and CH_4 emissions over 163 d of liquid dairy manure storage. However, this study saw no difference between 10% and 20% inoculum. The reason for this difference is unclear, although it could be due to differences in fresh manure or in inoculum microbial abundance due to age, storage conditions, or manure characteristics (Habtewold et al. 2018a).

The highest emissions were from inoculated, gradually-filled tanks. As already discussed, both inoculum presence and gradual-filling on average increased emissions, therefore it follows that these tanks would be the highest producing. Inoculum presence and gradual-filling also reduces the ratio of substrate to microorganisms, which leads to higher emissions. Higher concentrations of substrate will increase the rate of microbial degradation, creating an excess of VFAs and reducing the pH of manure. The observed pH varied little between tanks, though the pH of the 0% batch tank was slightly lower compared to the other tanks. On a farm scale, the effects of gradual filling may be greater, as fresh manure is added in comparatively smaller amounts, more

frequently. On the other hand, laboratory research which uses batch filling with or without inoculum, may underestimate emissions compared to farm-scale emissions.

3.5. Conclusion

This study used 11.4 m^3 tanks to study the effect of gradual vs batch filling on manure storage tanks with 0%, 10% and 20% inoculum. Our results show that tanks containing inoculum emit more total CO_2-eq GHGs when filled gradually. Both CH_4 and NH_3 emissions were highest in gradually-filled tanks with inoculum, while N_2O did not exhibit any clear relationship with fill type. Higher manure temperature and lower substrate/microbe ratio were key factors which might have contributed to these higher emissions in gradually-filled tanks. For both fill-types, tanks without inoculum produced the least CH_4, N_2O, and NH_3. This resulted in 24% fewer total CO_2-eq emissions when no inoculum was present. Our results suggest that batch-filling experiments underestimate emissions compared to gradual filling.

4. Dairy manure acidification reduces CH_4 emissions over short and long-term

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4.1. Introduction

Stored liquid dairy manure produces large amounts of methane (CH_4), a greenhouse gas (GHG) which has 28× the global warming potential of CO_2 on a 100-yr time horizon (Pachauri and Mayer 2015). Practices that reduce CH_4 production include acidification of fresh manure (FM) to reduce the pH of microbial community (Petersen et al. 2012; Habtewold et al. 2018b; Shin et al. 2019) and complete cleaning manure storages during emptying to remove any chance of residual manure inoculating FM (Ngwabie et al. 2016; Habtewold et al. 2018a). Complete removal of residual manure, however, is difficult and currently no management practices are known to approach this problem. Acidifying residual manure following tank emptying may disrupt its inoculating effect, and also reduce the pH of FM added to the tanks. Additionally, there is no information about long-lasting effects of acidification and hence no information on the inoculating ability of residual, previously acidified manure.

Acidification has been implemented in several European countries primarily to reduce ammonia emissions from swine manure, but has also shown good results in CH_4 reduction (Kai et al. 2008; Fangueiro et al. 2009). Methanogenesis is disrupted following acidification by making the environmental conditions less hospitable for methanogens (Habtewold et al. 2018b). Sulfuric acid (H_2SO_4) is a preferred acid, because it causes additional methanogenesis inhibition from

sulfide production (Petersen et al. 2012; Habtewold et al. 2018b; Shin et al. 2019). Limited research, however, has looked at dairy manure acidification with H_2SO_4. Berg et al (2006) reported up to 90% CH_4 reductions using lactic acid (pH <5), while Petersen et al (2012) reported 66% reduction of CH_4 from cattle slurry acidified using hydrochloric acid (pH <5) (Berg et al. 2006b). Recently, Sokolov et al. (2019) monitored meso-scale liquid dairy manure storages and measured 89% reductions in CH_4 emissions from H_2SO_4–acidified manure (pH 6). Currently, acidification is done by mixing acid into manure in temporary storages prior to being moved to regular storage (Kai et al. 2008). This requires extra infrastructure and a large quantity of acid. Thus, applying acid to residual manure following storage emptying to reduce emissions from subsequent stored FM may provide a cost-effective alternative of manure acidification.

Residual manure in emptied manure storages becomes an inoculum for the FM being added (Jayasundara et al. 2016). The presence of inoculant is hard to prevent in manure storages, as there is little value for farmers to clean tanks which continually receive animal waste. Current research shows that the presence of inoculum shortens the lag phase for methanogen population to adapt and produce CH_4 thus increasing overall emissions (Wood et al. 2014a; Jayasundara et al. 2016; Ngwabie et al. 2016). Although some research has explored reducing CH_4 emissions through better tank cleaning, a good practice for dealing with this inoculum has not been created (Massé et al. 2008; Jayasundara et al. 2016). Given that acidification can disrupt the activities of methanogen populations (Habtewold et al. 2018b), acidifying the residual manure in storage tanks might also disrupt its inoculating effect, while being cheaper and easier than fully cleaning manure storages or continuously acidifying all FM.

Additionally, no research has addressed the inoculating ability of residual manure which has been previously acidified. For example, if manure acidified in the summer of year one is stored

until spring of year two, it is unknown what the inoculating ability of the residue from previously acidified manure would be. Since acidification inhibits methanogens, it may also reduce inoculating capabilities. Reducing the inoculating ability of residual manure may increase the long-term treatment effects of acidification, and may avoid yearly acidification practices and hence reducing the cost and handling for farmers.

The objective of this study was to quantify CH_4 production at 17, 20, and 23°C and test the following hypothesis:

- There will be no difference in CH_4 emissions between the liquid dairy manure with no inoculum and the liquid dairy manure with freshly acidified inoculum.
- There will be no difference in CH_4 emissions between the acidified liquid dairy manure with inoculum and the liquid diary manure with freshly acidified inoculum.
- There will be a difference in CH_4 emissions between liquid dairy manure with previously acidified inoculum and liquid dairy manure with untreated inoculum.
- There will be a difference in CH_4 emissions between freshly acidified treatments and liquid dairy manure with untreated inoculum.

4.2. Methods

4.2.1. Treatments

This experiment had five acidification treatments, and three incubation temperatures (17, 20, and 23°C), performed in triplicate. Each experimental unit contained 150 mL of FM with varied acidification and inoculum. Treatments containing inoculum had 17% (30 mL) inoculum for a total volume of 180 mL. Inoculum level was within range of levels used in previous research (5-25%) (Ngwabie et al. 2016; VanderZaag et al. 2017). The acidification treatments are shown in Table 4.1: A – Control/FM with untreated inoculum; B – FM with inoculum acidified 6 months

prior; C – FM with inoculum acidified immediately prior to start of study with 0.03 mL of 98% H_2SO_4; D – FM acidified immediately prior to the start of the study with 0.03 mL of 98% H_2SO_4 with untreated inoculum; E – FM with no inoculum. To account for CH_4 production from inoculum, 150 mL of untreated inoculum, previously acidified inoculum, and acidified inoculum (0.15 mL of 98% H_2SO_4) were incubated in duplicate at each temperature (Table 4.1).

Table 4.1 Contents of each treatment which were anaerobically incubated at 17, 20, and 23°C in triplicate. Treatments are: A – FM + untreated inoculum; B – FM + inoculum manure acidified 6 months prior; C – FM + inoculum acidified immediately prior to the study with 0.03 mL of 98% H_2SO_4; D – FM acidified immediately prior to the study with 0.03 mL of 98% H_2SO_4 + untreated inoculum; E – FM without inoculum. Inoculums without FM were incubated: F – Untreated inoculum; G – Inoculum manure acidified 6 months prior.

	Treatment					**Inoculum Only**	
Volume (mL)	**A "control"**	**B**	**C**	**D**	**E**	**F**	**G**
FM	150	150	150	150	150	0	0
Untreated Inoculum	30	0	30	30	0	150	0
Inoculum Acidified 6 m prior	0	30	0	0	0	0	30
98% H_2SO_4	0	0	0.03	0.03	0	0	0

4.2.2. Manure and Inoculum Preparation

Inoculum manure was obtained from a Dalhousie University research site, Bio-Environmental Engineering Centre, Truro, NS, where both acidified and untreated manure were stored for 6 months in outdoor manure tanks (Sokolov et al., 2019). The acidified manure was treated in June 2017 at a rate of 2.4 mL acid L^{-1} manure using 70% H_2SO_4 (pH 6). The untreated manure had a pH of 7.4 during storage. Acidified and untreated manure was collected on October 7, 2017 (105 days after acidification) from the middle depth of the storage tanks and kept refrigerated (7°C) until the start of the trial. This manure is henceforth referred to as inoculum.

FM used was the liquid fraction of solid-separated raw dairy manure. It was obtained 3 d prior to start of the trial from a temporary storage (~37 m^3) adjacent to a 150 cow barn located on an

Ontario commercial dairy farm. More information about the farm and manure composition is described in Balde et al (2016) (Baldé et al. 2016a). All inoculums and FM were homogenised in large pails prior to filling digestion bottles. For treatments receiving acid prior to incubation (treatments C and D), inoculum or FM was measured out and mixed with acid in a beaker and allowed to react for 15 min. A preliminary test was done to find the amount of acid needed to reach pH 6 in the FM and the same quantity was used in the newly acidified inoculum (0.03 mL of 98% H_2SO_4). The pH was measured using a calibrated IQ 150 pH meter (Spectrum® Technologies, Inc., Aurora, IL) before manure was poured into prepared jars.

Triplicate subsamples of each type of inoculum were taken at the start of the trial for measurement of pH and analysis of total solids (TS) and volatile solids (VS). FM subsamples were taken per bottle by measuring out 50 mL more manure than needed per bottle and then then taking a 50 mL subsample. Samples were kept frozen (-10°C) until analysis. Following the end of the trial, the manure in each bottle was mixed, pH measured and further 50 mL subsamples taken for final TS and VS analysis.

4.2.3. Manure analysis

The TS and VS analysis was modified from Standard Methods 2540 B (American Public Health Association and Water Environmental Federation 2005) by weighing 25 mL of manure in a crucible of known weight and again after overnight drying in an oven (ISOTEMP® 255G, Fisher Scientific, Ottawa, ON) at 105°C. The dried manure samples were placed in a muffle furnace (ISOTEMP® 186A, Fisher Scientific, Ottawa, ON) at 550°C for 1 hr and weighed again to find the VS.

4.2.4. BMP Set-Up and Monitoring

Anaerobic digestion bottles (Bellco Glass Inc., Vineland, NJ) of 500 mL capacity, with re-sealing septa were filled with inoculum and FM. Inoculum was added first, followed by manure, and the two were not actively mixed together to reflect conditions in manure storages. Bottle headspace was purged with 70% N_2 and 30% CO_2 gas for 2 minutes to remove any O_2 present, and then sealed shut and incubated for 116 d in temperature-controlled incubation chambers (modified Coldspot Deluxe freezer, Sears, Hoffman Estates, USA; Hotpack 355381, SP Industries Company, Warminster, USA; Koolatron, Brantford, CA). The chambers were set to 17, 20, and 23°C with variability of ±1°C. These temperatures were chosen to represent a range of annual average temperature in temperate areas as used by the IPCC to estimate CH_4 emissions from manure storages (Dong et al. 2006). Two temperature probes (HOBO pendant, Onset Computer Corporation, Bourne, MA) were placed at two heights within each incubator to record hourly average temperature.

Biogas sampling was on average twice a week, depending on gas accumulation in headspace. Volume of gas built up in each bottle was measured using a water displacement manometer, which was made of 0.7 cm ID tubing filled with water. The volume of gas under the pressure exerted by the water was calculated by the equation for volume of a cylinder.

The volume of biogas under atmospheric pressure conditions was calculated by rearranging Boyle's Law:

$$\boldsymbol{Volume_{STP}} = \frac{\boldsymbol{Volume_P} \times (\boldsymbol{\rho} \times \boldsymbol{g} \times \boldsymbol{h} + \boldsymbol{P_{atm}})}{\boldsymbol{P_{atm}}} - \boldsymbol{Volume_0} \qquad \text{Eq. 4.1}$$

where $Volume_{STP}$ is the volume of biogas created at atmospheric pressure (mL), $Volume_0$ is the volume of atmospheric gas that was already present in the manometer prior to biogas entering,

$Volume_P$ is the total space the biogas and atmospheric gas take up under the pressure of the water (mL), P_{atm} is atmospheric pressure (Pa), and lastly, the water pressure is calculated by ρ is the density of water (g cm^{-3}), g is the force of gravity (cm s^{-2}), and h is the height of the displaced water column (cm).

Every two weeks 20 mL biogas samples were drawn from the headspace of each bottle and stored in evacuated glass exetainers. Samples were analysed for CH_4 and CO_2 concentrations using an Agilent 490 Micro-gas chromatograph (Agilent Technologies, Santa Clara, USA) at Agriculture and Agri-Food Canada, Lethbridge, AB. Emissions of CH_4 (L L^{-1}) were calculated by multiplying the biogas volume × CH_4 concentration (%).

CH_4 emissions from FM were calculated by subtracting the scaled emissions created by inoculum alone. Emissions were also scaled by VS (g kg^{-1}) to account of variability between bottles.

4.2.5. Data Analysis

Treatments effects were assessed for cumulative CH_4 L kg^{-1} VS, final VS kg, and final pH using a 2-way ANOVA by PROC GLM in SAS software (SAS Institute Inc., Cary, NC, USA), which uses ordinary least squares general linear model with a Sidak adjustment to control familywise error. Effect size was calculated using partial eta^2 (η_p^2). Significant results were followed up with a post hoc Sidak groupings comparison set at a significance of $p < .05$.

The Gompertz equation, a sigmoidal regression model was used to describe the cumulative CH_4 production. The methods used followed VanderZaag et al (2017), modified from Browne et al (2013) and Kafle and Kim (2013). The equation was:

$$G(t) = G_0 \times exp\left\{-exp\left[\frac{R_{max} \times \exp(1)}{G_0} \times (\lambda - t) + 1\right]\right\} \quad \text{Eq. 4.2}$$

Where G(t) was the cumulative CH_4 production at time t (L CH_4 kg^{-1} VS), G_0 was the maximum CH_4 potential (L CH_4 kg^{-1} VS d^{-1}), λ was the CH_4 production lag phase (d), and t was the time (d) of incubation.

4.3. Results and Discussion

4.3.1. pH

The initial pH values of the FM and inoculum are shown in Table 4.2. The pH of the fresh liquid manure was 7.57 while all inoculums had lower pH values (6.2 – 6.4). Initial pH appeared to be a poor predictor of CH_4 production. For example, FM with no inoculum (treatment E, 7.57) produced the least total CH_4 at all temperatures and had the highest initial pH (scaled average).

Table 4.2 The average pH of the FM (FM) and inoculum and the average pH scaled by volume of inoculum and FM (30:150 mL) for each treatment at the start of the trial and the average pH for each treatment and temperature at the end of the trial (after 116 d).

	Trial start		Trial end		
Treatment	FM	Inoculum	17°C	20°C	23°C
FM with untreated inoculum	7.57	7.44	7.82±0.03	7.93±0.01	8.12±0.03
FM with previously acidified inoculum	7.57	6.44	7.61±0.03	7.83±0.02	8.09±0.02
FM with acidified inoculum	7.57	6.28±0.05	7.85±0.03	7.93±0.01	7.99±0.11
Acidified manure with untreated inoculum	6.96±0.01	7.44	7.79±0.05	7.93±0.01	8.06±0.03
FM with no inoculum	7.57	-	7.57±0.06	7.86±0.01	8.13±0.02
Inoculum control	-	7.44	7.73±0.05	7.77±0.02	8.03±0.07
Previously acidified inoculum control	-	6.44	7.57±0.08	7.78±0.05	7.90±0.04

The final manure pH measured after 182 d of incubation varied little between treatments, although there was a statistical difference due to temperature ($p<0.0001$, $\eta_p^2 = 0.8531$). The full results of the 2-way ANOVA are summarised in Table 4.3. The average pH was 7.73±0.03 at

17°C, 7.90±0.01 at 20°C, and 8.08±0.02 at 23°C. This pH increase also corresponds with increases in CO_2 and CH_4 emissions, which suggests this increase was likely due to increased biological reactions at higher temperatures increasing the pH at a faster rate. Increase in pH over time in stored untreated and acidified manure has been previously observed (Wood et al. 2012; Sokolov et al. 2019b).

4.3.2. Gompertz model

The Gompertz equation had previously shown to be acceptable for modelling CH_4 emissions from manure and waste (Kafle and Kim 2013; Browne et al. 2013; VanderZaag et al. 2017). The model had a good fit with treatments that produced CH_4, with all $r^2>0.85$, although the model failed at 17°C and 20°C for low CH_4 producing treatments (Table 4.4).

4.3.3. Treatment Effects

Results of 2-way ANOVA on total CH_4 g kg^{-1} VS are shown in Table 4.3. There was a significant effect due to treatment ($p<.0001$) and temperature ($p<.0001$), but no effect due to interaction of treatment and temperature ($p=0.4557$).

Table 4.3 Results of 2-way ANOVA, effects of treatment, temperature (temp), and interaction of treatment and temperature (treatment*temp) for final pH and cumulative CH_4 L kg^{-1} VS. Showing mean squared error (MSE), degrees of freedom (df), F-value (F), p-value (p), eta-squared (η_p^2), and 90% confidence intervals (CI_{90}).

Data	Source	MSE	df	F	*p*	η_p^2	CI_{90}	
Final pH	Treatment	0.0228	4	4.37	0.0067	0.0686	0.0000	0.1362
	Temp	0.4545	2	87.1	<.0001	0.0686	0.5245	0.7558
	Treatment*Temp	0.0217	8	4.16	0.0019	0.1305	0.0000	0.1306
CH_4 g kg^{-1} VS	Treatment	10791	4	18.9	<.0001	0.4155	0.1661	0.5199
	Temp	19492	2	34.1	<.0001	0.3753	0.1654	0.5059
	Treatment*Temp	572.50	8	1.00	0.4557	0.0441	0.0000	0.0000

4.3.3.1. Inoculant Effects

Below 23°C, the only treatment which produced substantial quantities of CH_4 was the control – FM with untreated inoculum (Figure 4.1), which produced significantly ($p<.05$) more total CH_4

than all other treatments (68% more, average of all temperatures). As expected, FM without inoculum produced the least amount of CH_4 (Treatment E: 19.5±9.30 L CH_4 kg^{-1} VS), which was 81% less than the control. At 23°C, a lag of 11 d for the control and 67 d for FM without inoculum were obtained using the Gompertz model. This 56–d difference represents the time for the methanogen populations to establish and start producing substantial amounts of CH_4. Following the lag, however, at 23°C both treatments needed relatively similar time to reach 50% (16 and 19 d, respectively), 75% (13 and 12 d, respectively), and 90% (11 and 9 d, respectively) of its total CH_4 production. This indicates a similar rate of increase in CH_4 production following the lag phase in both treatments with different daily CH_4 emissions (R_{max} was 3.69 vs 1.45 at 23°C). These results follow what previous authors have shown, in which inoculum increases CH_4 production by reducing the production lag phase (Wood et al. 2014a; Jayasundara et al. 2016; Ngwabie et al. 2016).

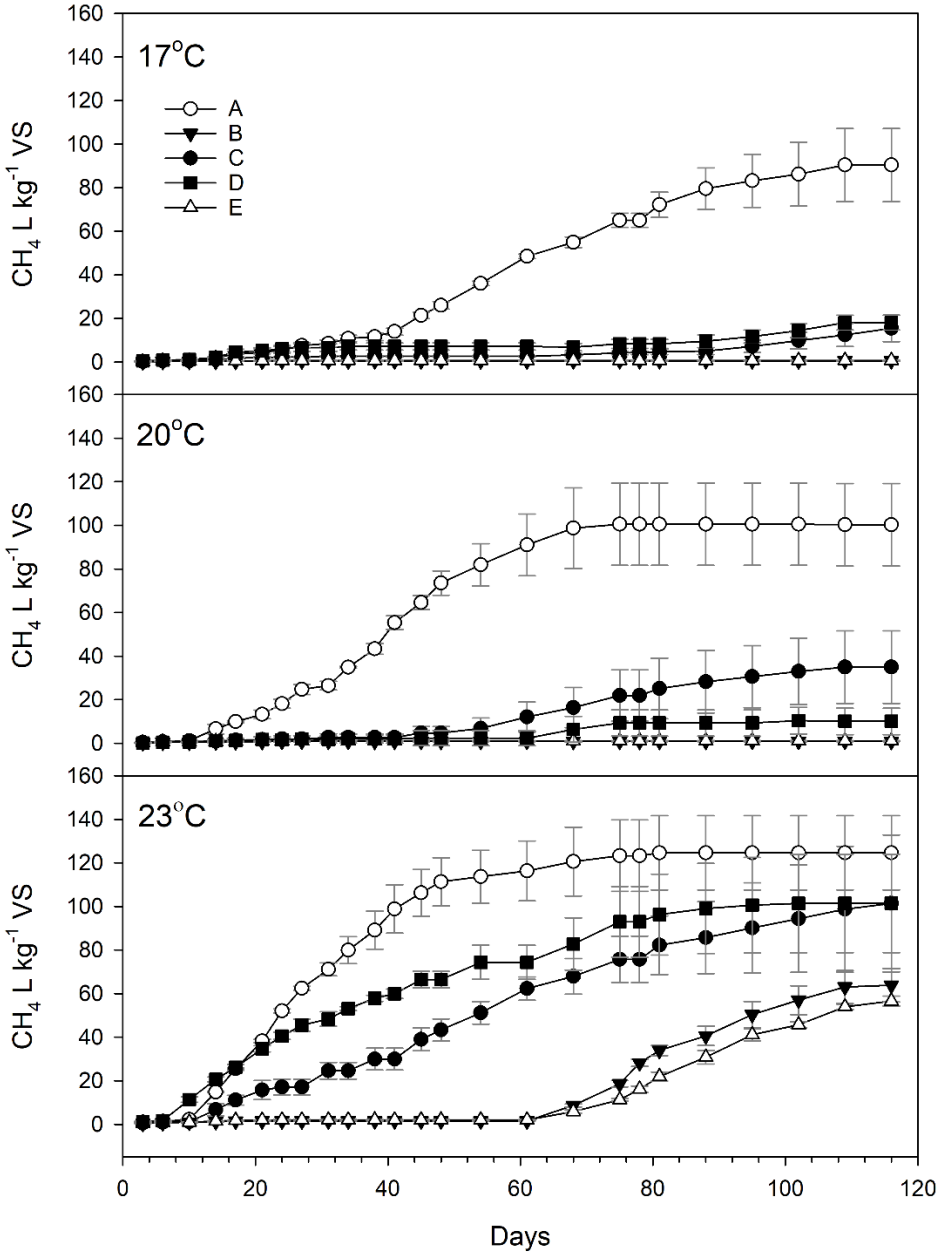

Figure 4.1 Cumulative methane production (L CH_4 kg^{-1} VS) from each treatment at 17°C, 20°C, and 23°C. Black fill symbols denote acid-added treatments, while white fill had no acid. Treatments are denoted by letters: A – FM with untreated inoculum; B – FM with inoculum acidified 6 months prior; C – FM with acidified inoculum; D – acidified FM with untreated inoculum; E – FM with no inoculum. Error bars show standard errors of the mean.

4.3.3.2. Effect of Inoculum that was Acidified 6-months Prior

FM with previously acidified inoculum and FM with no inoculant produced consistently the least amount of CH_4 with very similar total CH_4 emissions (average over all temperatures 21.8±10.7 vs 19.5±9.30 L CH_4 kg^{-1} VS, respectively). These two treatments also produced similar emission

curves, and similar Gompertz model parameters (Figure 4.1 and Table 4.3, respectively). This similarity suggests that previous acidification (6 months prior) of the inoculum had long-term effects of disrupting the methanogen communities to the extent of removing the inoculating effect on FM during this incubation period. Habtewold et al (2018) measured significant reductions in methanogen activity in acidified manure with corresponding 76 – 78% overall reductions in CH_4 from 120 d of dairy manure storage. This reduced methanogen activity is likely the reason for the lack of an inoculating effect.

At 17°C and 20°C both FM with previously acidified inoculum and FM with no inoculum produced in total <1.5 L CH_4 kg^{-1} VS (Table 4.4). This was less than the total CH_4 produced in the first 10 d of incubation at any temperature from the control. At 23°C both treatments increased CH_4 production after >60 d lag (64 d and 67 d, respectively; Table 4.4) and reached cumulative emissions of 63.8±7.64 and 56.6±2.29 L CH_4 kg^{-1} VS, respectively. Due to the low production at 17°C and 20°C, the time to reach 50% of the B_o was <20 d for both treatments, while at 23°C this increased to >80 d.

Table 4.4 Measured total methane production compared to the estimated total methane production (G_o), lag phase (λ, days), and maximum daily production (R_{max}, L CH_4 kg VS^{-1}) using the Gompertz equation. Treatments are denoted by letters: A – FM with untreated inoculum; B – FM with inoculum acidified 6 months prior; C – FM with acidified inoculum; D – acidified FM with untreated inoculum; E – FM with no inoculum.

	Measured B_0 L CH_4 kg VS^{-1}			Gompertz model parameters								
				G_0, L CH_4 kg VS^{-1}			λ, d			R_{max}		
Treatment	17°C	20°C	23°C	17°C	20°C	23°C	17°C	20°C	23°C	17°C	20°C	23°C
A	90.4±16.8	100.3±18.9	124.7±17.1	101	101	125	30.0	30.0	10.7	1.48	1.48	3.69
B	0.59±0.16	0.91±0.20	63.8±7.64	0.55	0.91	68.1	0.00	0.00	63.7	0.02	0.05	1.82
C	15.5±6.08	35.0±16.6	101.4±31.5	-	41.6	113	-	40.0	14.7	-	0.60	1.30
D	18.1±3.36	10.1±6.11	101.5±22.6	-	13.4	104	-	25.5	1.97	-	0.15	1.61
E	0.71±0.15	1.13±0.12	56.6±2.29	0.71	1.08	65.3	0.00	∞	66.6	0.04	0.07	1.45

4.3.3.3. Acidification

The two newly acidified treatments differed in the location of the acid addition, although both received the same amount of acid per bottle (0.03 mL 98% H_2SO_4). The scaled average initial pH (inoculum and FM) was slightly higher in the FM with acidified inoculum compared to acidified FM with untreated inoculum (7.18 vs 7.10). The slight difference in CH_4 production was not statistically significant, therefore we assume that the location of acidification makes no difference on CH_4 reduction (Figure 4.1). Currently acidification is done by collecting FM in temporary tanks where small batches are acidified prior to being pumped into larger storage. Given our results, the same CH_4 reductions may be achieved by adding acid to residual manure following storage emptying rather than to FM prior to each filling event. This would reduce the need for additional infrastructure and manure handling. However, it is important to note that gradual filling, as it occurs on farms, may change the treatment effectiveness compared to the batch filling done in this study as additional manure would increase the pH, especially with the high buffering capacity of dairy manure.

The acidified treatments produced on average 52% and 59% less CH_4 emissions compared to the control, with also different emissions curves (Figure 4.1). This is consistent with results from Petersen et al (2012) who used hydrochloric acid at pH <5 and had 66% reduction in CH_4 production from cattle slurry (no specified temperature). Reductions at 17°C (83% and 80%) also align with results from Sokolov et al (2019), who had 85% reduction from dairy slurry at pH 6.5 at 12-20°C monitored from June to October.

Interestingly, the lag phase at 23°C was short, especially for acidified FM with untreated inoculum, which had a lag of 2 d (Treatment D; Table 4.4). This might be a result of acidification of FM rather than the inoculum, which had a lag of 15 d, suggesting the acidified

FM may not have had an initial impact on methanogenesis and reduced CH_4 emissions only observed following a longer contact time with the acidified FM. Comparatively, the control had a lag 11 d, although it produced markedly more CH_4 than the acidified treatments.

The newly acidified treatments were not statistically different from the FM with no inoculant and FM with previously acidified inoculant, although on average they produced more total CH_4. At 17°C the newly acidified treatments produced on average 96% more CH_4, at 20°C they produced 95% more CH_4, and at 23°C they produced 41% more CH_4 (L kg^{-1} VS, Figure 4.1). Therefore, although acidification was able to reduce CH_4 emission by >50%, removing inoculum was a more effective treatment method. It is important to note, however, that removing inoculum is very difficult on farms, as residual manure is always present following tank emptying and acidification has been shown to reduce CH_4 emissions by up to 89% over several month (June to October at 17-20°C) (Sokolov et al, 2019). Therefore, acidification has the potential to be a better treatment.

4.3.3.4. Temperature

Temperature was a good predictor of CH_4 production for all treatments, although the CH_4 did not increase in a correspondingly linear fashion, with markedly more CH_4 produced at 23°C. On average, the CH_4 production was 25.1±9.46 L CH_4 kg^{-1} VS at 17°C, 29.5±10.9 L CH_4 kg^{-1} VS at 20°C, and 89.6±9.99 L CH_4 kg^{-1} VS at 23°C. Average CH_4 production at 23°C was statistically different from production at 17°C and at 20°C (p<.05), although no statistical difference was found between 17°C and 20°C.

The total amount of CH_4 produced from the FM with untreated inoculum at all temperatures was less than the IPCC estimated CH_4 from liquid dairy manure (Dong et al. 2006). The IPCC default dairy emissions are 240 L CH_4 kg^{-1} VS (Table 10A-4, Dong et al., 2006). The results from this

study were 63%, 58%, and 48% less than the IPCC estimates at 17, 20, and 23°C, respectively (Table 4.4). Although the total CH_4 produced from FM with untreated inoculum was low in this study, it was within the range reported by Massé et al (2016) of 85.4 – 158.5 L CH_4 kg^{-1} VS measured from dairy manure incubated at 20°C (Massé et al. 2016).

4.4. Conclusion

Acidification of liquid dairy manure, added to inoculum or to FM, reduced CH_4 production by 81% at 17°C, 78% at 20°C, and 19% at 23°C compared to the untreated control. Acidifying the inoculum rather than the FM could reduce the need for extra infrastructure on farms, and therefore be used when tanks are emptied rather than each time they are filled. Using inoculum that was acidified 6 months prior reduced CH_4 production by 99% at 17°C and 20°C, and 49% at 23°C compared to the control. This effect was similar to the treatment without inoculum, indicating that acidifying residual manure after emptying may be an option to eliminate inoculum-induced CH_4 emissions when manure storages cannot be completely emptied. This suggests that acidification may have continued treatment effects more than one year after being added, by disrupting the inoculating ability of residual acidified manure. Reducing the number of times farmers would need to add acid, while still reducing CH_4 emissions could make acidification easier and cheaper for farmers. Lastly, CH_4 from acidified manure and acidified inoculum treatments was similar at 17°C or 20°C, but increased dramatically (81%) at 23°C. In the future, field-scale research and testing smaller acidification rates is needed to determine the most cost-effective treatment for liquid dairy manure.

5. Greenhouse gas mitigation through dairy manure acidification

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5.1. Introduction

Dairy farm manure storages are important sources of greenhouse gases (GHGs), especially with increasing production and a Canadian commitment to reducing national GHG emissions by 30% from 2005 emissions (Environment and Climate Change Canada 2012). The predominant emissions from liquid dairy manure systems are methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3) (Le Riche et al. 2017). Both CH_4 and N_2O are significant GHGs, which have 28× and 265× the global warming potential of carbon dioxide (CO_2) (Pachauri and Mayer 2015). Ammonia, on the other hand, is an indirect source of N_2O emissions following atmospheric deposition of nitrogen on surfaces (Dong et al. 2006; Pachauri and Mayer 2015). Although a number of mitigation strategies presently exist, many require significant infrastructure (e.g. anaerobic digesters) or create possible trade-offs through increasing one GHG in lieu of another (e.g. solid-liquid separation reduces CH_4 while increasing N_2O emissions) (Alexander 1977; Janzen 2008; Guest et al. 2017; Fillingham et al. 2017). Acidification is a possible treatment option, because it can inhibit methanogenesis and also limit NH_3 volatilization (Clemens et al. 2002).

To date acidification has shown promising results in reducing GHGs from manure storage systems. Methane emissions have been reduced through acidification by 17% to 90%, depending

on the slurry type, acid used, and target pH (Berg et al. 2006a; Berg and Pazsiczki 2006; Petersen et al. 2012; Fangueiro et al. 2015), while NH_3 emissions have been similarly reduced by 40% to 98% (Lefcourt and Meisinger 2001; Shi et al. 2001; Berg et al. 2006b; Kai et al. 2008; Wang et al. 2014; Fangueiro et al. 2015). Most manure acidification research, however, has mostly been conducted in Europe and more specifically in Denmark, where 2011 legislation banned the surface application of livestock manure, unless acidified below a pH of 6.4 (Nyord et al. 2013). Acidification also poses possible health concerns to farmer and livestock due to handling of strong acids and volatile sulfur compounds such as hydrogen sulfide (H_2S) (Borst 2001; Regueiro et al. 2016). Because of these issues it is important to study acidification on a meso-scale prior to farm testing and with smaller acid doses which would be less potentially harmful.

Swine manure, however, differs from dairy manure by having different levels of organic matter (OM) and nutrients, which changes the emissions being produced and the buffering reactions occurring (Agriculture and Agri-Food Canada 1980). Therefore, results from swine slurry acidification cannot be fully extrapolated to dairy systems. Additionally, other manure acidification research has been performed only in laboratory-based studies, which involve small manure volumes incubated in controlled climatic conditions (Fangueiro et al. 2015). This cannot be directly extrapolated to on-farm manure tanks that have large volumes of manure and changing environmental conditions. A need therefore exists to expand research to larger scales of study using dairy manures to increase the ability to extrapolate results to whole farm emission reductions.

The objective of this research was to quantify the overall reduction in GHG emissions from liquid dairy manure acidified with sulphuric acid (H_2SO_4) during storage using replicated meso-

scale manure storage systems. We also considered estimated acid costs relative to possible carbon credits due to GHG emission reductions.

5.2. Methods

5.2.1. Site description

The research site was located at Dalhousie University's Bio-Environmental Engineering Centre (BEEC) in Bible Hill, Nova Scotia, Canada (45°45' N, 62°50' W). It contained 6 in-ground, meso-scale rectangular manure storage tanks (1.8 m deep, 6.6 m^2). Each tank was enclosed under separate flow-through, steady-state chambers (~13 m^3 headspace) constructed of an aluminium frame and 6 mil greenhouse plastic. This replicated manure storage system has been previously described by VanderZaag et al. (2010) and Wood et al. (2012). The chambers created an enclosed environment where air was continuously drawn across the manure surface at a rate of ~0.5 m^3 s^{-1} from the inlet opening to the exhaust fan. This created laminar air flow within the chambers with about 2 full air exchanges per minute (Wood et al. 2012; Le Riche et al. 2017). This air flow rate (Q) was measured using cup anemometers (Davis Instruments, Hayward, CA) within the venturi outflow ports of each tank and 1 min averages were recorded by a CR1000 datalogger (Campbell Scientific Inc, Logan, UT). Gas samples were drawn from both the inlet and outlet ports (Livingston and Hutchinson 1995; Rochette and Hutchinson 2005; Wood et al. 2014a; Le Riche et al. 2017). The measured average gas concentrations from the samples and the hourly averaged air flow were used for flux calculations.

All tanks were emptied and cleaned prior to the start of the trial and then batch filled once with raw liquid dairy manure (June 8, 2017) to a depth of 1.6 m. Liquid manure was obtained from a local dairy farm, which housed about 100 cows in a tie-stall facility which used washed quarry sand as bedding material. Manure was pumped out of an outdoor circular tank on the farm.

Efforts were made to gather manure as fresh as possible, although due to spring manure spreading, there was some mixing with old manure.

Manure was acidified on June 24, 2017 and then stored through December 1, 2017 (160 d monitoring period). The experimental treatments included three rates of acidification: medium pH (1.4 mL L^{-1}), low pH (2.4 mL L^{-1}), and control (no acidification). A preliminary lab-based acidification trial was performed to establish the quantity of acid required for each treatment. Treatments were assigned to the tanks in two blocks, to account for spatial variability and edge effects. As the tanks were all in a single order, the first block was the first 3 tanks in the row and the second tank was the last 3 tanks. Tanks not receiving acid, received 20 L of water through the same application process.

5.2.2. Acidification

Technical grade, 70% sulphuric acid (H_2SO_4) was purchased from a local supplier (Bebbington Industries, Dartmouth, NS). The grade and strength was chosen to allow for safer application with lower concentration and better price value of product. Undiluted acid was added to the manure once to minimize handling. Acid was pumped out of 20 L containers using a peristaltic pump (Masterflex Easy-Load II, Gelsenkirchen, DE) and 7.6 m acid-resistant tubing (9.7 mm ID, Masterflex C-Flex tubing Gelsenkirchen, DE). The tubing was attached to an aluminum pole that was of sufficient length to reach the full depth of the manure tank. Acid was pumped slowly (~1 L min^{-1}) as the pole was moved throughout the tank to mix the acid into the manure as evenly as possible. The process took 15-30 min per tank.

5.2.3. Environmental and manure measurements

Manure samples from each of the tanks were collected 5× throughout the study period. Each sample was a composite of 18 sub-samples (9 locations at tank bottom and mid-depth) and

samples were frozen until analysis. Manure was analysed for total solids (TS), volatile solids (VS), total ammonical-nitrogen (TAN), total nitrogen (TN), and pH, at the Nova Scotia Department of Agriculture's Provincial Soils Lab in Bible Hill, NS. Additional samples were taken at 2 depths per tank (80 and 150 cm), as a composite of 9 locations. These were analysed for TS and VS at Agriculture and Agri-Food Canada in Ottawa, ON.

Manure pH and temperature measurements were collected directly within the tanks 4× throughout the study period by inserting a calibrated pinpoint tip ion-sensitive field-effect transistor probe at 4 depths (2, 20, 40, 80 cm) and 6 locations (total 24 readings per tank) using FieldScout pH 400 meter (Spectrum Technologies, Aurora, IL, USA).

Air temperature within each tank was measured at 20 cm above the manure surface using shielded copper/constantan (type-T) thermocouples and recorded by a CR3000 datalogger (Campbell Scientific Inc., Logan, UT, USA). To simulate rainfall, 80 L (12 mm) of water was applied to each tank twice weekly using sprinklers. This rainfall amount was based on daily average 30-yr normal rainfall data for the region (Environment and Climate Change Canada 2011).

5.2.4. Flux measurements

5.2.4.1. Methane and Nitrous Oxide

Air samples were continuously drawn from the chambers through polyethylene tubing into two tunable diode lasers. Every 30 s a sub-sample was taken from a different chamber, as well as two ambient locations at the chamber inflow. The TGA100A trace gas analysers (Campbell Scientific, Inc., Logan, UT, USA) measured CH_4 and N_2O concentration of each sample. The average CH_4 and N_2O flux densities (Flux, mg m^{-2} s^{-1}) were calculated every 4 min based on the following:

$$\boldsymbol{Flux} = \frac{\boldsymbol{C_{out} - C_{in}}}{\boldsymbol{A}} \times \boldsymbol{Q} \qquad \text{Eq. 5.1}$$

where C_{IN} is the concentration measured at the inflow (mg m^{-3}), C_{OUT} the concentration at the outflow, A is the surface area (m^2), and Q is the airflow rate (m^3 s^{-1}). The 24 h mean emissions were calculated from the 4 min measurements.

5.2.4.2. Ammonia

Ammonia concentrations were measured at 24 h deployment intervals 3× weekly using a gas washing technique (VanderZaag 2010; Wood et al. 2012). Samples from each tank and two ambient inflows were pumped through dispersion tubes in 0.125 L of 0.005 mol L^{-1} phosphoric acid (H_3PO_4). The total volume of air pumped through each trap over the 24h deployment was ~1.9 m^3. The air flow for each event was monitored using in-line flow meters (Actaris Metering Systems, Greenwood, SC, USA). Following deployment, loss of volume due to evaporation was filled with fresh acid and a sample of the acid was taken to Agriculture and Agri-Food Canada, Ottawa, ON for quantification of NH_3-N using the QuikChem® Method 12-107-06-2-A modified for 0.005 mol L^{-1} H_3PO_4 matrix using a Lachat QuikChem FIA+ Q8500 Series (Hofer 2003). The time averaged NH_3-N concentrations were calculated by:

$$\boldsymbol{C_{NH3,air}} = \frac{\boldsymbol{C_{NH3_{aq}}} \times \boldsymbol{V_i}}{\boldsymbol{V_{air}}} \qquad \text{Eq. 5.2}$$

where $C_{NH3,\ air}$ (mg m^{-3}) is the concentration of NH_3-N in the air subsample, $C_{NH3,aq}$ (mg L^{-1}) is the aqueous concentration of NH_3-N in the acid solution, V_i (0.125 L) is the volume of liquid in the acid trap, and V_{air} (m^3) is the volume of air that passes through the acid during deployment.

5.2.5. Data analysis

For each treatment the total GHG emissions were converted to 100-yr CO_2-eq values to compare treatments on the basis of their global warming potential. The total CO_2-eq GHG emissions were calculated by summing the CO_2-eq converted totals of CH_4, direct N_2O, and indirect N_2O due to NH_3. Global warming potentials used for converting CH_4 and N_2O were 34 and 298, respectively (IPCC 2014). The amount of indirect N_2O from NH_3 was calculated using the IPCC emission factor of 0.01 (Dong et al. 2006).

The methane conversion factors (MCFs) were calculated using IPCC methods using VS from composite manure samples gathered prior to acidification (analysis described above). The VS mass was calculated using manure density of 1050 kg m^{-3} and the B_o used in the MCF calculation was 0.24 m^3 CH_4 kg^{-1} VS (Dong et al. 2006).

For better comparison of treatments, N_2O and NH_3 emissions were scaled by the mass of TN and TAN in the tank, based on composite manure samples gathered prior to acidification.

Statistical differences were calculated on monthly sums (Jun to Oct) using a monthly repeated measures multi-level linear model analysis using Proc Mixed in SAS (SAS Institute Inc., Cary, NC, USA). All parameters were fixed and model fit was disregarded. Significance was considered when $p<0.05$.

5.3. Results and Discussion

5.3.1. Manure and Environmental Conditions

5.3.1.1. Temperature

The monthly air temperature in the chambers was on average 20℃ in July and August, gradually declining to 10.5℃ in the first two weeks of December. The average ambient air temperature

throughout the study was ±1°C of the 30-yr normal for the region except for September and October which were on average +1.8°C and +2.8°C warmer than the 30-yr normal, respectively.

Manure temperatures showed a depth gradient, with up to a 10°C difference between the top and bottom, depending on the time of year and day. The average manure temperature in all tanks was 20°C in July and August and 12°C in October. The average manure temperature between tanks varied by < 2°C.

5.3.1.2. pH

In the first two weeks following acidification, manure pH values were variable, with areas of low (<4) and unaltered pH (6.5-7) values in each tank. After two weeks, however, the pH values became more uniform. On July 16, the medium pH treatment manure was on average pH 6.5, the low treatment manure was pH 6, and the control average pH manure was pH 7. Over the entire monitoring period following acidification the control tanks had consistently the highest pH (average pH 6.5 – 7.9), the medium pH tanks ranged from 6.1 – 6.8, and the low pH tanks remained with the lowest average pH and ranged from 6.1 – 6.7. The addition of more acid, with the low pH compared to the medium pH treatment, did not further reduce the manure pH. The low pH tanks received 40% more acid, but their pH was only half a value lower than the medium pH treatment.

Following pH becoming uniform, distinct depth pH gradients developed in all manure tanks, including the controls (Figure 5.1). All tanks had a higher pH near the manure surface, with pH decreasing with depth (Figure 5.1). The largest gradient was in the control manure tanks, especially in August and October. The pH depth gradient is likely due to NH_3 and CO_2 emissions closer to the surface.

All tanks showed an increase in pH over the 160 d monitoring period. This trend had been observed in other studies as well (Wood et al. 2012). The control tanks had a pH increase from 6.8 (July 23) to 7.8 (Oct 29) (Figure 5.1). The largest pH change occurred in the top manure layer. The medium treatment increased from pH 6.3 to pH 6.7 and the low treatment from pH 6.1 to pH 6.6. The increase in pH is likely due to the cumulative effect of NH_3 and CO_2 losses from the uppermost manure layer. It was expected that acidification would reduce the NH_3 volatilization and hence decrease the pH change over time. Our results support this, as the control tanks had the largest pH increase over time.

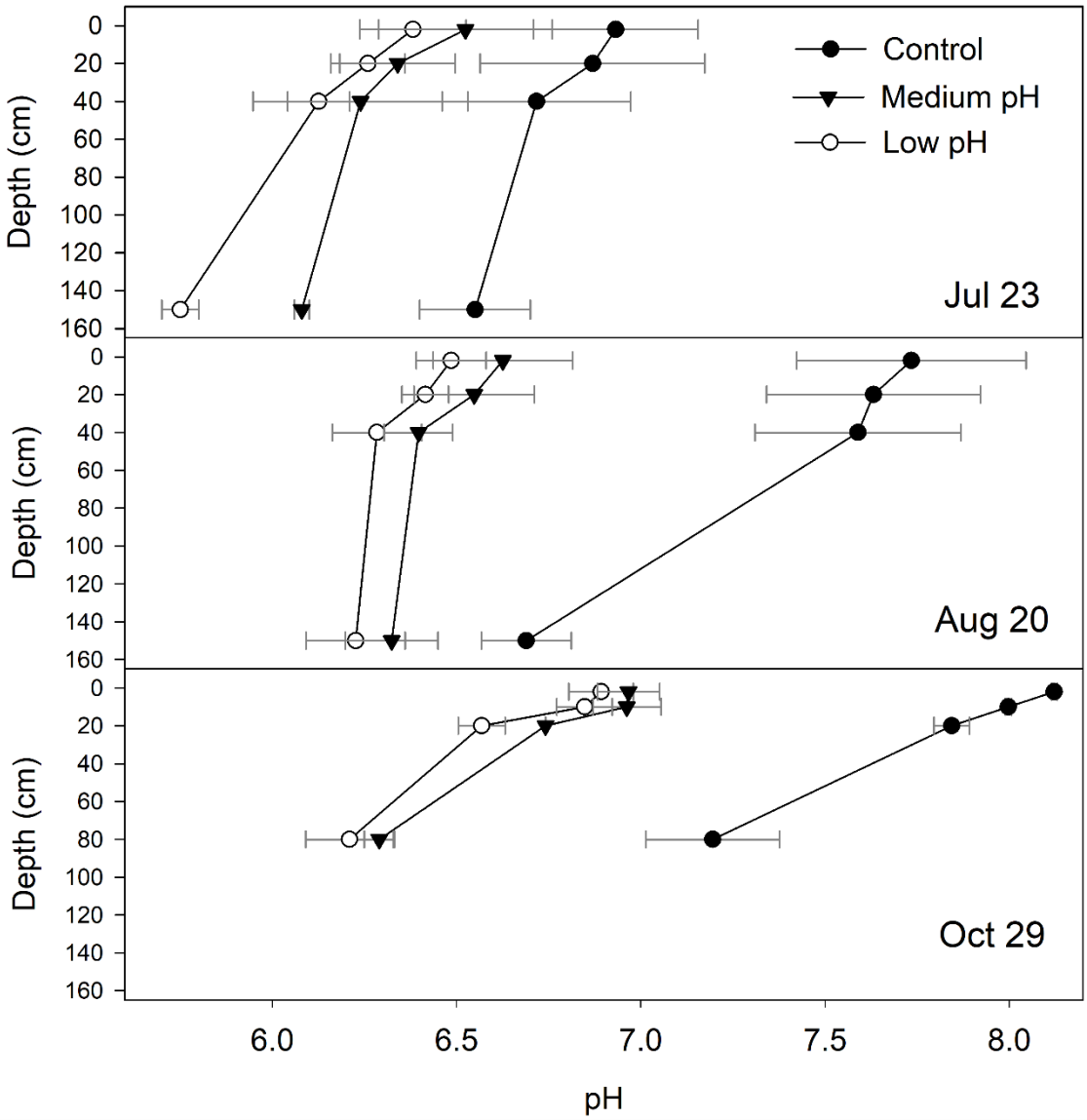

Figure 5.1 Average dairy manure pH of control, medium pH (1.4 mL 70% H_2SO_4 L^{-1}), and low pH (2.4 mL 70% H_2SO_4 L^{-1}) treatments across different depths (cm) on Jul 23 (29 d after acidification), Aug 20 (57 d), and Oct 29 (127 d). Error bars represent standard error of means.

5.3.1.3. Manure characteristics

At the start of this trial, the manure had an average TS of 21.5% and all treatments were within ±5% of the average (Table 5.1). The average VS content, however, was 9.6%, which is more than 3× higher then reported by Le Riche et al. (2017) who monitored manure from the same farm – this difference is likely due to the positioning of the intake pipe used by the tanker truck that obtained manure at the farm and delivered it to the research site. Additionally, manure used

in this study was taken from the farm tank later in the spring, after manure had been already pumped out and spread onto fields. The high TS means that >50% of the manure solids were inorganic, which can be explained by the large quantity of sand bedding which is mixed into the manure at the farm where manure was obtained.

There was limited manure surface crusting observed in all tanks, which has previously been observed for sand bedding manure (Le Riche et al. 2017). The limited crusting suggests that there were few low density solids that floated to the top and the majority settled to the bottom.

The TN and TAN at start of the trial were 0.5% and 0.2%, respectively, decreasing over the monitoring period (Table 5.1). Le Riche et al (2017) reported 0.16% TN and 0.09% TAN from manure taken from the same farm, which is markedly less than our results.

Previous research observed higher final VS, TS, and TN in acidified manure compared to untreated manure (Schils et al. 1999; Sørensen and Eriksen 2009; Fangueiro et al. 2010, 2013). Sorensen and Eriksen (2009) attributed higher TN in acidified dairy manure to be due to inhibited microbial decomposition of N-containing organic compounds, since they found the ratio of TN:TAN to be unchanged between treatments. The results of the present study found overall average TS, VS, and TN decreased less in the acidified manure compared to untreated manure, although the difference was not substantial (Table 5.1).

Table 5.1 Manure characteristics for the control, medium pH (1.4 mL 70% H_2SO_4 L^{-1}), and low pH (2.4 mL 70% H_2SO_4 L^{-1}) treatments including acid added (L), pH, total solids (TS, %), volatile solids (VS, %), nitrogen (%), and ammonium-N (%) at the start of the trial (June 18), following acidification (July 16), and at the end of the trial (December 8).

	Control	Medium pH	Low pH
70% H_2SO_4 added (L m^{-3} manure)	—	1.4	2.4
		pH	
Trial Start	7.4	7.4	7.4
Following Acidification	6.9	6.6	5.9
Trial End	7.3	6.6	6.5
		TS (%)	
Trial Start	21.6	22.5	21.7
Following Acidification	16.5	17.3	15.0
Trial End	12.1	13.5	12.9
		VS (%)	
Trial Start	8.6	9.7	9.5
Following Acidification	8.2	9.3	8.2
Trial End	5.5	6.5	6.6
		Nitrogen (%)	
Trial Start	0.45	0.48	0.45
Following Acidification	0.37	0.49	0.41
Trial End	0.33	0.39	0.39
		Ammonium-N (%)	
Trial Start	0.19	0.19	0.18
Following Acidification	0.13	0.20	0.17
Trial End	0.14	0.17	0.19

5.3.2. Emissions

5.3.2.1. Methane

The cumulative CH_4 emissions for the entire study period (160 d) were 3640, 491, and 388 g m^{-2} for the control, medium pH, and low pH treatments, respectively (Table 5.2). Previous research using manure from the same farm had reported cumulative CH_4 emissions of 4508 g m^{-2} (over 173 d of continuous monitoring) (Le Riche et al. 2016). Our control results were within 20% of previous results for a similar length of monitoring (per unit area). The daily average CH_4 fluxes

were 22.6, 3.05, and 2.41 g m^{-2} d^{-1} from control, medium pH, and low pH treatments, respectively.

The control manure tanks had a lag phase with max emissions occurring 45 d from tank filling (Figure 5.2). The controls exhibited an emission curve observed in previous studies, coinciding with maximum air temperatures reached mid-August (VanderZaag et al. 2009; Le Riche et al. 2016). This was followed by a slow decline in the following months. The warmer weather in September and October extended the decreasing emission phase in the fall, with unseasonably high fluxes observed until the end of monitoring (December 1).

The acidified tanks had no lag phase and did not follow the typical emission curve for CH_4 production (Figure 5.2). The highest CH_4 emissions were observed at the onset of the monitoring period and decreased logarithmically till the end. This suggests a substantial portion of the CH_4 emitted may have been the physical off-gassing of CH_4 already present in the manure. It also means there was no seasonal response to temperature in the acidified tanks, unlike the control tanks. This is important because it suggests that the duration of storage and temperature had no effect on the treatment ability of acidification.

Table 5.2 Methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3) emissions from stored dairy manure control, medium pH (1.4 mL 70% H_2SO_4 L^{-1}), and low pH (2.4 mL 70% H_2SO_4 L^{-1}) treatments from June 24, 2017 to December 1, 2017 (160 d) expressed as cumulative (g m^{-2} and g m^{-3}) and mean daily (g m^{-2} d^{-1}) emissions, as well as scaled cumulative CH_4 expressed as methane conversion factor (MCF) and scaled cumulative N_2O and NH_3 emissions by total nitrogen (TN, g kg TN^{-1}) and total ammoniacal-nitrogen (TAN, g kg TAN^{-1}).

	Control pH	Medium pH	Low pH
VS (kg $tank^{-1}$)	958	1077	1053
TN (kg $tank^{-1}$)	50.1	52.9	50.1
TAN (kg $tank^{-1}$)	21.2	21.2	20.0
		CH_4	
Cumulative (g m^{-2})	3640±784	491±71.8	388±89.4
Cumulative (g m^{-3})	2275	307	243
Mean daily (g m^{-2} d^{-1})	22.6	3.05	2.41
CH_4 (kg $tank^{-1}$)	24.1	3.26	2.57
CH_4 (m^3)	36.8	5.0	3.9
CH_4 (g kg^{-1} VS)	25.2	3.02	2.44
B_o*VS	230	258	253
MCF	0.16	0.019	0.016
		N_2O	
Cumulative (g m^{-2})	1.27±0.63	0.31±0.02	0.53±0.61
Cumulative (g m^{-3})	0.79	0.19	0.33
Mean daily (g m^{-2} d^{-1})	0.008	0.002	0.003
(g kg TN^{-1})	0.17	0.04	0.07
(g kg TAN^{-1})	0.42	0.10	0.17
		NH_3	
Cumulative (g m^{-2})	641±1.97	376±2.03	304±14.9
Cumulative (g m^{-3})	401	235	190
Mean daily (g m^{-2} d^{-1})	3.94	2.31	1.86
(g kg TN^{-1})	85.2	47.2	40.2
(g kg TAN^{-1})	203	118	101

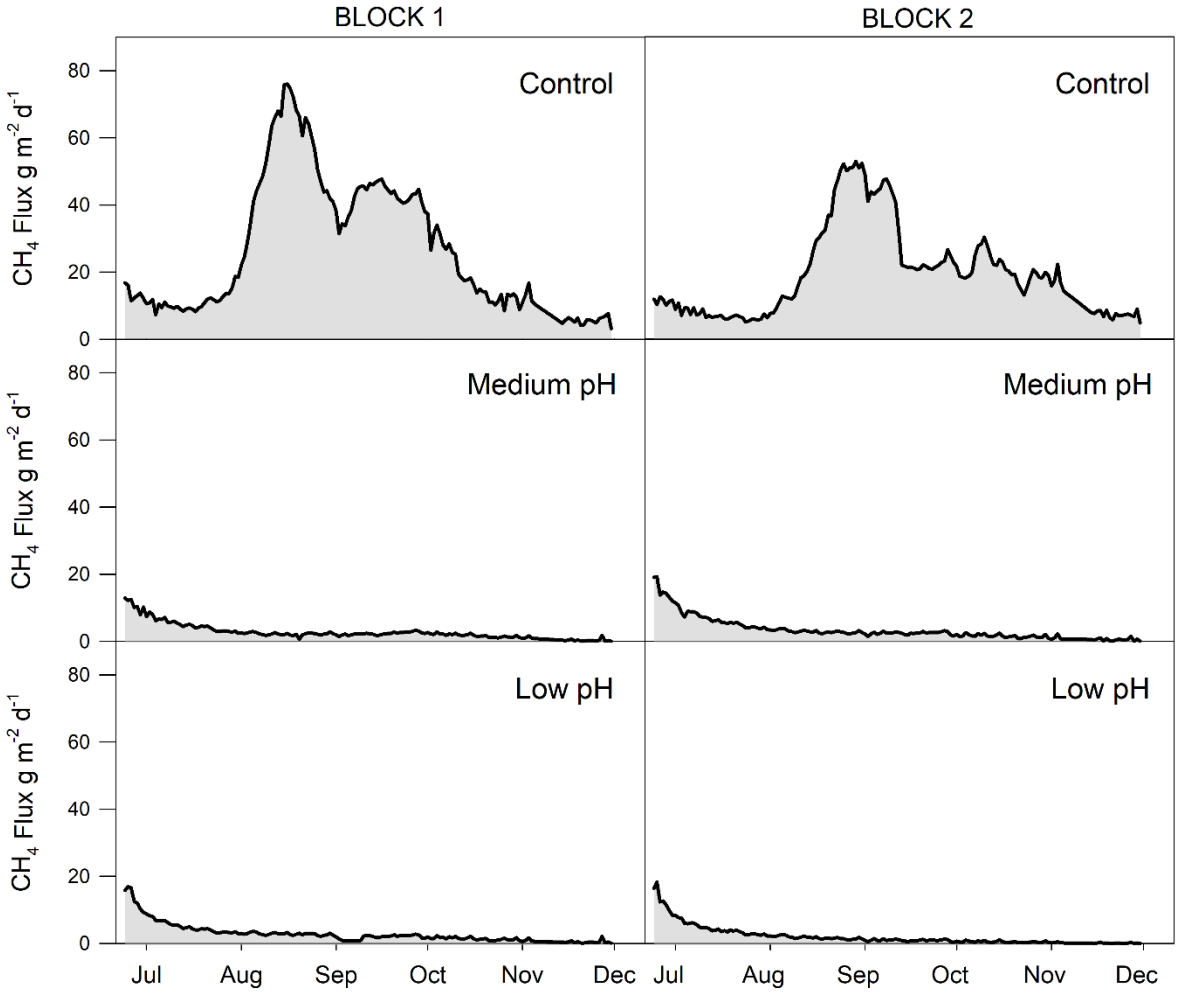

Figure 5.2 Daily average methane (CH_4) emissions (g m^{-2} d^{-1}) from each tank over the entire storage monitoring period (June 24 to December 1, 2017, 160 d) for the control, medium pH (1.4 mL 70% H_2SO_4 L^{-1}), and low pH (2.4 mL 70% H_2SO_4 L^{-1}) treatments.

A daily temperature response was evident in all treatments. Control tanks had maximum CH_4 production between 0700 h and 0800 h which, as previously shown, is caused by bubbles bursting due to evolution of the boundary layer in the morning (Wood et al. 2013). All acidified treatments had maximum emissions between 1200 h and 1300 h and minimum between 2000 h

and 2200 h (Figure 5.3).

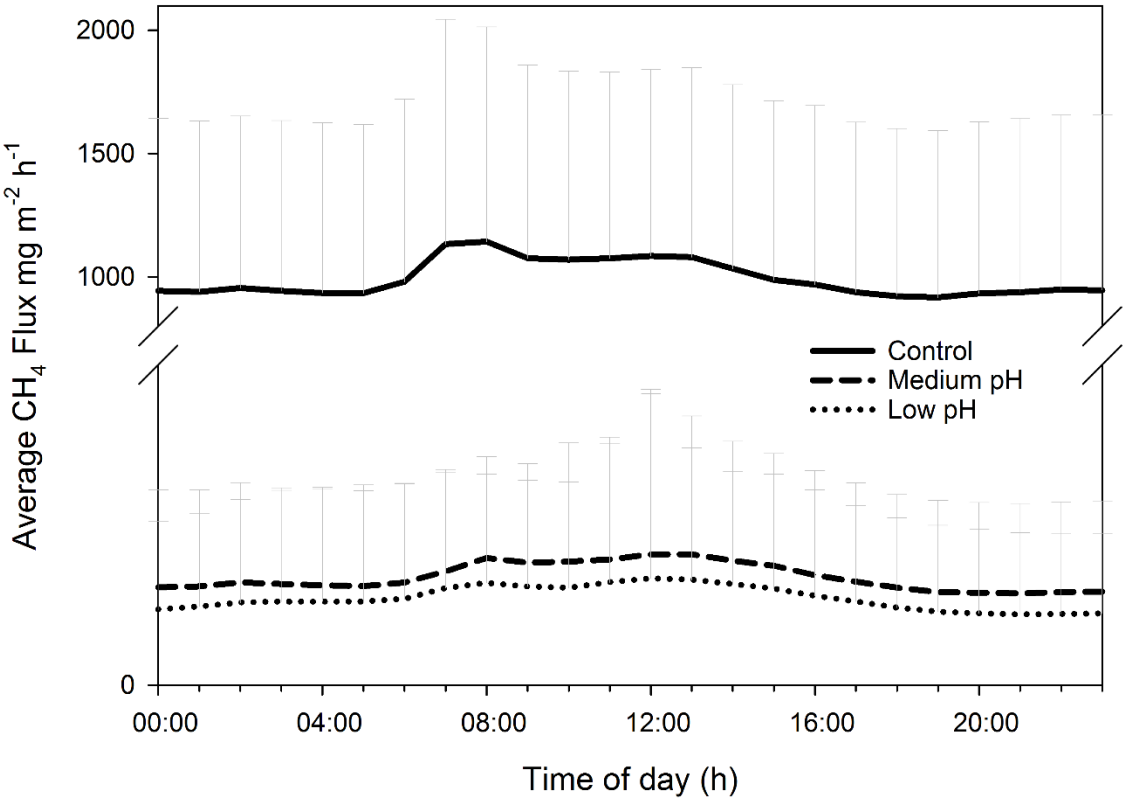

Figure 5.3 Methane (CH_4) emissions averaged over the entire study (June 24 – October 30, 2017) by time of day (h) for each treatment (control, medium pH, and low pH). Whiskers are showing standard deviation.

The pattern of CH_4 production in the acidified tanks suggests that there was CH_4 production occurring, but at much lower rates compared to the control tanks. In fact, Habetwold et al (2018) found that the methanogen abundance was reduced by 6% and the activity by 20% in the same acidified manure. They suggest that acidification inhibits, rather than kills the methanogens present in manure (Habtewold et al. 2018a).

Methane emissions from medium and low pH treatments did not differ (P = 0.3935). Compared to the control, cumulative emissions were statistically different and reduced by 87% and 89% in the medium (P=0.0331) and low (P=0.0188) pH treatments, respectively (Table 5.2). The MCF

of control tanks was on average 0.16 (16%) which was markedly lower than values from previous research using the same manure (Table 5.2). Le Riche et al. (2016) reported a MCF value of 0.30. This is likely due to the high TS and VS content in our study. Manure high in TS/VS have previously been shown to have lower CH_4 emissions, compared to more dilute manures (Massé et al. 2003; Vedrenne et al. 2008). The acidified tanks (at both pH levels) had MCF values almost 1/10 of the control (0.02 or 2%).

5.3.2.2. Nitrous Oxide

The cumulative N_2O emissions over the entire study period (160 d) were 3.32, 0.82, and 1.40 g m^{-2}, from control, medium pH, and low pH, respectively (Table 5.2). There was no statistical difference between treatments ($P>0.05$).

Le Riche et al (2017) measured N_2O emissions from manure with wood shavings as bedding which formed a surface crust. They found N_2O emissions of 11.5 g m^{-2} which is up to 10× that of manure with sand bedding. The low emissions from manure with sand bedding are due to lack of crust formation on top of manure, which creates an environment for nitrification (VanderZaag et al. 2009; Le Riche et al. 2017). The absence of crust is a more influential factor than pH level on the emission of N_2O.

The daily average N_2O fluxes were 0.021, 0.005, and 0.009 g m^{-2} d^{-1} from control, medium pH, and low pH tanks, respectively (Table 5.2). The control tanks showed increasing N_2O fluxes from the start of July to mid-August, followed by a steep decline through to the end of the monitoring period (Figure 5.4). The acidified tanks did not show such trends, with the exception of the second low pH treatment replicate, which showed higher emissions than the other acidified tanks.

The reduction of N_2O in the acidified tanks is important, as many mitigation strategies will reduce CH_4, while increasing N_2O (i.e. manure covers, aeration). Avoiding GHG trade-offs means that overall GHG will be reduced and make for a better treatment option.

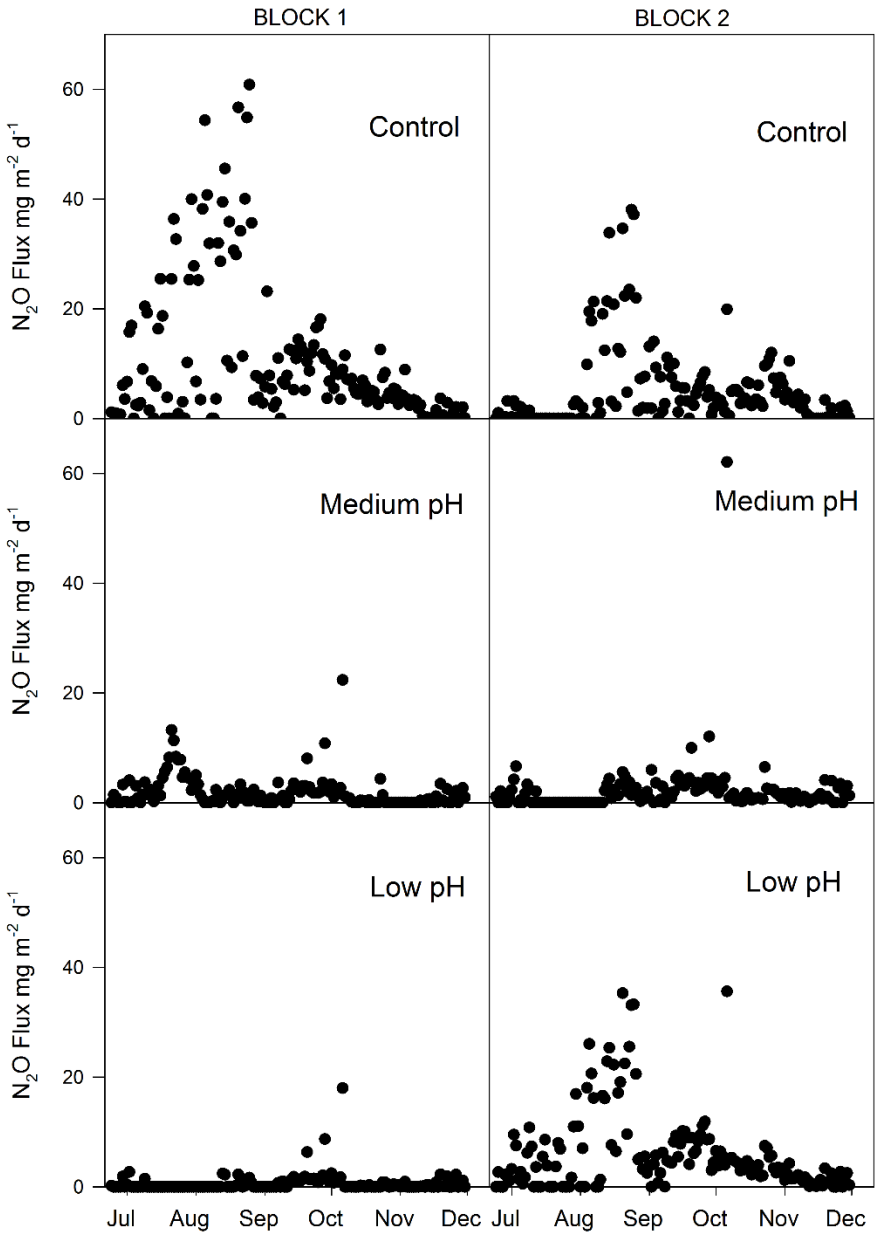

Figure 5.4 Daily average nitrous oxide (N_2O) emissions (mg m^{-2} d^{-1}) from each tank over the course of the entire storage monitoring period (June 24 to December 1, 2017, 160 d) for the control, medium pH and low pH treatments.

5.3.2.3. Ammonia

The cumulative NH_3 emissions for the 160 d of monitoring were 641, 376, and 304 g m^{-2} for the control, medium pH, and low pH tanks, respectively (Table 5.2). Our results are similar to Ngwabie et al. (2016), who reported on average NH_3 emissions of 450 g m^{-2} from stored liquid dairy manure (182 d) with 0 – 25% inoculum.

The average daily NH_3 fluxes were 3.94, 2.31, and 1.86 g m^{-2} from control, medium pH, and low pH tanks, respectively. There was no significant difference between low and medium pH treatments ($P=0.2085$). However, there was a significant acidification treatment effect compared to untreated manure, where emissions were reduced by 41% for the medium pH ($P<0.0171$) and 53% in the low pH ($P<0.0085$; Figure 5.5). Additionally, the variability between treatment replicates was small (<5%).

Following acidification, the NH_3 emissions dropped considerably compared to the untreated manure and then NH_3 emissions increased and decreased following a seasonal temperature trend. From July to September the NH_3 emissions gradually increased in all tanks, with pH showing a corresponding increase in the low pH and control treatments (Figure 5.5). The low pH treatment increased from pH 6 to 6.4 from July to September and the control from pH 6.8 to 7.2. The medium pH, however, changed very little, remaining around 6.5 throughout. The NH_3 emissions from the medium pH treatment did not remain constant as the pH, but also followed the seasonal emission trend. By the end of the monitoring period, the low pH and medium pH treatments had similar pH ~ 6.5 (Figure 5.5).

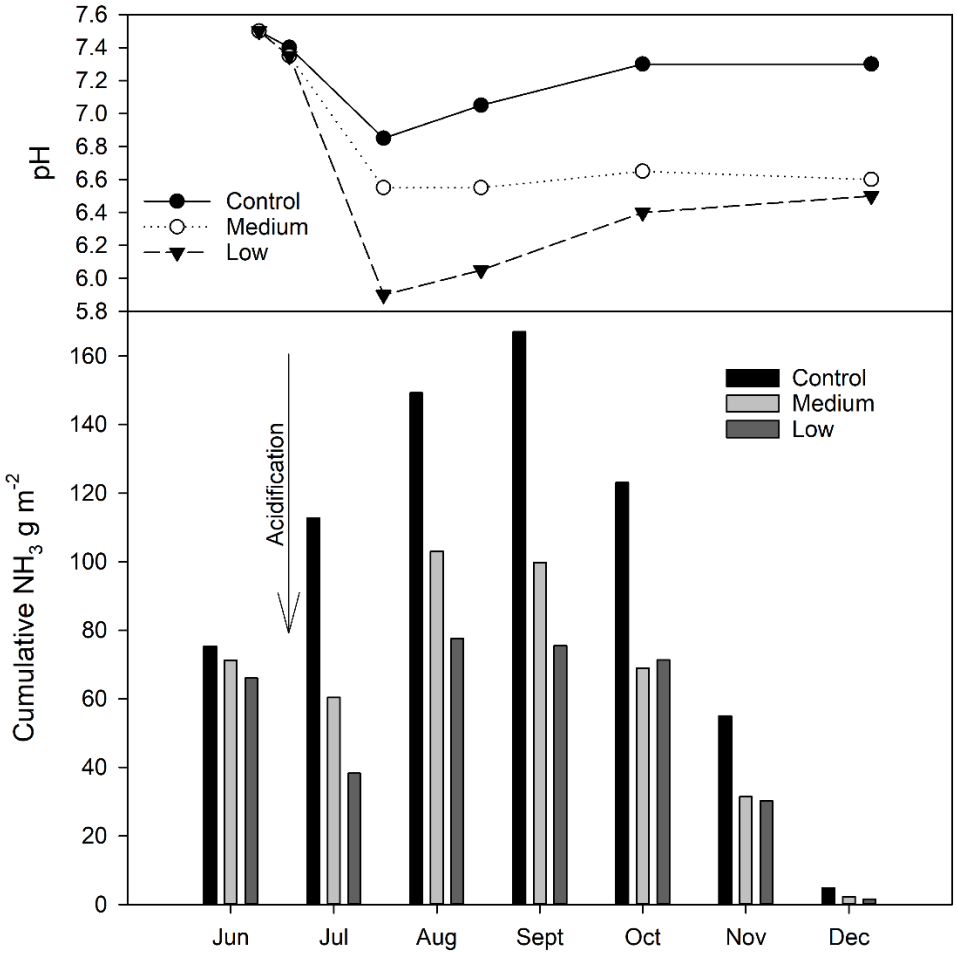

Figure 5.5 Monthly cumulative ammonia (NH_3) emissions (g m^{-2}) and average pH from each tank for the control, medium pH (1.4 mL 70% H_2SO_4 L^{-1}), and low pH (2.4 mL 70% H_2SO_4 L^{-1}) treatments. Month of June includes June 10-23 to represent emissions prior to acidification, while the remaining time period are monthly average from July 1 to December 8, 2017.

5.3.2.4. CO_2-equivalent emissions

Overall, the medium pH treatment reduced total CO_2-eq emissions by 85%, while the low pH treatment reduced emissions by 88% (Table 5.3) compared with the non-acidified manure (control). For all treatments CH_4 made up the majority of the total CO_2-eq emissions. Control and medium pH treatments had 97 % CH_4 reductions. This is consistent with Le Riche et al. (2016, 2017) who reported 98% of CO_2-eq emissions were from CH_4 for manure from the same farm. The remainder of the total emissions in the control and medium pH treatments was from

direct and indirect N_2O emissions, which were <4% of the total CO_2-eq. In the low pH treatment, direct and indirect N_2O emissions made up 12% of total CO_2-eq emissions due to the lowered CH_4 emissions (86%).

Table 5.3 Total greenhouse gas emissions (kg CO_2-eq m^{-2}) over the 160 d monitoring period for methane (CH_4), nitrous oxide (N_2O), and indirect N_2O from ammonia (NH_3) for control, medium pH (1.4 mL 70% H_2SO_4 L^{-1}), and low pH (2.4 mL 70% H_2SO_4 L^{-1}) treatments, presented on a CO_2 equivalent (CO_2-eq) basis. The proportion (%) of each greenhouse gas contribution are also presented.

	Control		Medium pH		Low pH	
	(kg CO_2-eq m^{-2})	% of Total	(kg CO_2-eq m^{-2})	% of Total	(kg CO_2-eq m^{-2})	% of Total
CH_4	91.0	96.5%	12.3	97%	9.7	86%
N_2O – direct	0.4	0.5%	0.1	1%	0.2	1%
N_2O – indirect	3.0	3%	1.8	2%	1.4	13%
Total	94.4	100%	14.1	100%	11.3	100%

5.3.3. Cost analysis

As a preliminary assessment, the cost of acidification was calculated for use on a 150-cow dairy farm based on GHG emissions and manure production measured by Baldé et al (2016). We assumed using the medium pH acidification rate employed in this study (1.01 L 98% H_2SO_4 m^{-3} manure). The farm produced 25 m^3 of manure per day with annual emissions of 33 Mg CH_4 (Baldé et al. 2016b), or 1122 Mg on a CO_2-eq basis. Although only CH_4 was measured by Baldé et al (2016), we measured >96% of CO_2-eq emissions from liquid dairy manure are from CH_4. The annual acid cost was calculated assuming this daily manure production over 6 months. Since manure is usually spread in early spring and late fall, the manure stored over winter does not need acidification since Canada has low CH_4 emissions during the cold season. Therefore, only manure stored over the warm season would require any treatment. To account for possible price ranges, the cost of H_2SO_4 used was estimated at 3 price points, CAD \$200 Mg^{-1}, CAD \$400 Mg^{-1}, and CAD \$600 Mg^{-1} (Figure 5.6).

The acid cost was estimated to be CAD \$1700 yr^{-1}, CAD \$3390 yr^{-1}, and CAD \$5090 yr^{-1} at rates of CAD \$200 Mg^{-1}, CAD \$400 Mg^{-1}, and CAD \$600 Mg^{-1}, respectively. These rates would translate to CAD \$11.3 cow^{-1} and CAD \$22.6 cow^{-1} and CAD \$33.9 cow^{-1}, respectively, given that acidification occurs only during half the year when the weather is warm.

Carbon credit earnings were calculated by assuming 85% reductions in GHG emissions, based on the medium pH treatment in this study. Three possible carbon credit rates were used, given that the current rate is CAD \$10 Mg^{-1} CO_2-eq and is expected to increase to CAD \$30 Mg^{-1} CO_2-eq by 2020, and then to CAD \$50 Mg^{-1} CO_2-eq by 2022 (Environment and Climate Change Canada 2017b). The estimated earning at the 3 carbon credit rates, for 85% reduction from 33 Mg CH_4, were calculated to be CAD \$9,990, CAD \$29,960, and CAD \$49,930 (Figure 5.6). Since many farms produce less CH_4 due to management or environmental factors, we also calculated the three carbon credit rates for 85% reduction from 16 Mg CH_4. These were calculated to be CAD \$4,840, CAD \$14,530 and CAD \$24,210. Figure 5.6 shows the amount of savings at three acid prices for each carbon credit rate, given CH_4 total production of 33 Mg or 16 Mg. In most cases the carbon credits were more than the cost of acid, with potential for the farmer to make profit of CAD \$1,450 - \$48,230. Only in one scenario was there a cost of acid, which was Can\$250 yr^{-1}.

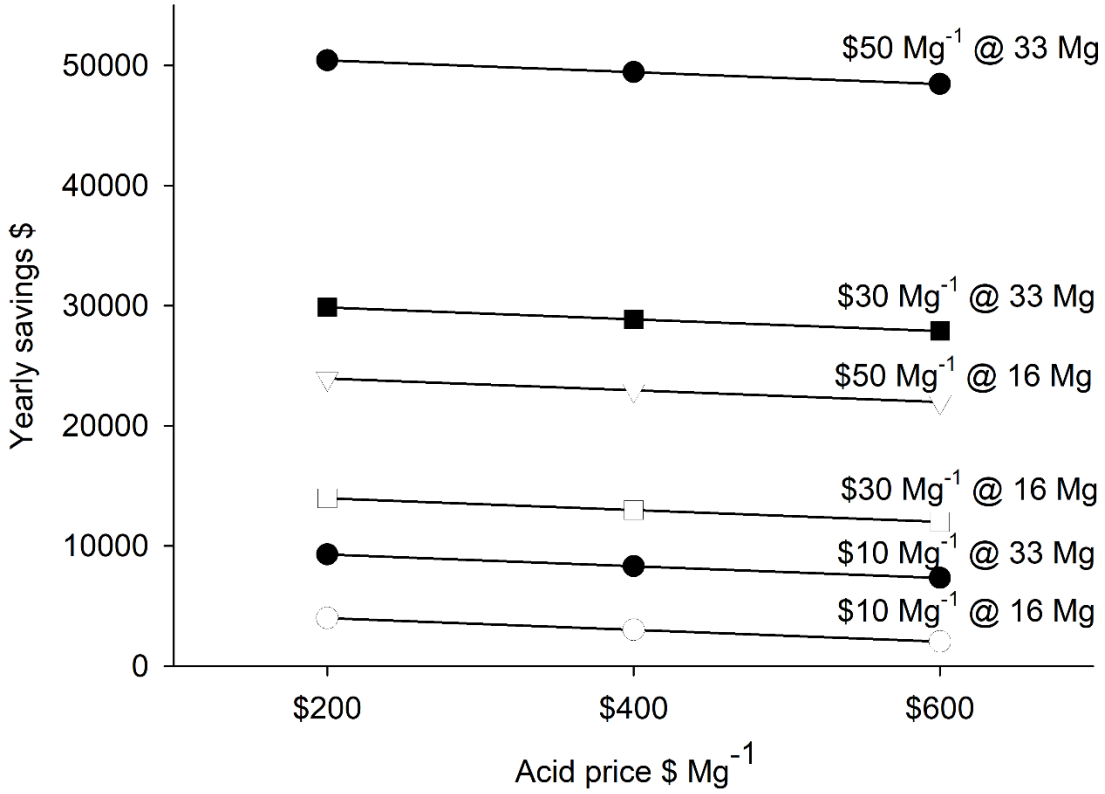

Figure 5.6 Annual savings (CAD$) against the acid price ($ Mg^{-1} 98% H_2SO_4) for each carbon credit rate of $10 Mg^{-1} CO_2-eq, $30 Mg^{-1} CO_2-eq, and $50 Mg^{-1} CO_2-eq from a farm producing 33 Mg and another producing 16 Mg of CO_2-eq GHG.

This simplified cost analysis offers insight, showing the possible environmental and financial benefits of manure acidification. However, the limitations of this analysis need to be highlighted. It does not include the cost of applying acid such as infrastructure, labour, and issues with bulk purchase of acid (e.g. shipping and storage costs). Additionally, each dairy farm will have different acid requirements based on the operational pH of their manure and also on the buffering capacity of their raw manure. Lastly, our study was performed by batch loading the manure storages and acidified in batch, which would be different from farms where tanks are gradually loaded and therefore incremental acidification is necessary. More research is necessary to find the most cost-effective way to apply acid on a farm-scale

5.3.4. On-farm acidification considerations

In assessing the cost benefits of manure acidification the approach we chose was a one-time acidification to a batch filled storage. This is similar to the continuous acid application currently used in Europe (Kai et al. 2008; Nyord et al. 2013), in that, a temporary tank is batch filled with manure scraped from barn floors and then acidified one-time. The manure is eventually pumped into longer-term storage, unlike our system where the manure stays in one storage the whole period. Our study does not account for continuous tank filling and manure removal, which might change the treatment ability of the acidification and also increase the cost of acid. More research is necessary to develop the best acid application method, which is easy and cost-effective for farmers. Also, there may be benefit to acidifying only once per year, or only acidifying inoculating manure at the bottom of emptied tanks, to delay CH_4 production until seasonal temperatures drop. And lastly, more acid rates need to be evaluated to find the rate that has the most benefit at the lowest cost.

5.4. Conclusion

Acidification of liquid dairy manure to pH 6.5 and 6.0 (compared with untreated manure with a pH of 7.0), in batch filled storage tanks, reduced overall GHG emissions by 85 and 88%, respectively. The largest contribution was from CH_4, which was reduced by 87% and 89%, respectively. For NH_3, there was a clear treatment effect, where emissions were reduced by 41% for the medium pH and 53% in the low pH compared to the non-acidified manure. A theoretical cost analysis using an example farm calculated that medium pH treatment would cost the farmer CAD \$1,700 - \$5,090 yr^{-1} in acid, but have the potential to be off-set by carbon credits received of at least \$4,800. In the future, acidification needs to be tested at lower rates to find the best cost-benefit acidification rate, include H_2S monitoring to ensure acidification is safe, as well as

testing in gradually filled tanks, and on dairy farms to see the effects of acidification on a larger scale and with continuous filling.

6. Acidification of residual manure in liquid dairy manure storages

6.1. Introduction

Liquid dairy manure is a substantial source of methane (CH_4) and moderate source of nitrous oxide (N_2O), and ammonia (NH_3) (Le Riche et al. 2016; Sokolov et al. 2019b). Both CH_4 and N_2O are greenhouse gases (GHG) contributing to global warming and climate change, while NH_3 is an indirect source of N_2O and is a toxic gas hazardous to human health (Jayasundara et al. 2016; Sokolov et al. 2019b). Liquid manure is often stored on farms for >100 d prior to spreading onto fields. During this storage period considerable amounts of GHGs and NH_3 are emitted to the atmosphere (Jayasundara et al. 2016).

Dairy manure acidification (to pH 6 – 6.5) with sulfuric acid (H_2SO_4) was found to reduce CH_4 (>87%) and NH_3 (>40%) emissions (Sokolov et al. 2019b). Sommer et al. (2017) reported 68% reductions of CH_4 and 62% of NH_3 with H_2SO_4 acidification (to pH 5.2 – 5.5). Kavanagh et al. (2019) reported 96% reductions of CH_4 and 85% of NH_3 with H_2SO_4 acidification (to pH of 5.5). The mechanism of CH_4 reduction is still unclear, as H_2SO_4 and pH reduction can disrupt microbial communities throughout all the processes of organic matter degradation as well as methanogens directly (Habtewold et al. 2018b). Habtewold et al. (2018) reported a methanogen reduction of 6% in abundance and 20% in activity between untreated and acidified dairy manure but observed no difference in the microbial communities. This suggests that H_2SO_4 primarily disrupts methanogenesis rather than other microbial processes, however, more research is necessary to confirm these results. Petersen et al (2012) reported substantial methanogen inhibitions (63 – 67%) from cattle slurry using potassium sulfate with no corresponding pH reduction. They suggest that sulfur transformations inhibit methanogenesis independent of any

pH reduction. Therefore, lower rates of H_2SO_4 may reduce CH_4 production without necessarily aiming for a certain manure pH value.

Due to the cost of acid, infrastructure and equipment, there is a need to make manure acidification more feasible. Treating only the inoculum (manure remaining in storage tanks after emptying) has been suggested to reduce the quantity and the frequency of acidification (Sokolov et al. 2020). As storages are difficult to completely empty, the residual manure becomes an inoculum for incoming fresh manure and increases subsequent CH_4 emissions by 34-52% (Ngwabie et al. 2016). If the inoculation process can be disrupted, then reductions can be expected (Sokolov et al. 2020). Sokolov et al. (2020) measured CH_4 production from manure incubated with 6-month-old, previously acidified inoculum and with 6-month-old, newly acidified manure inoculum. They reported 82% and 63% CH_4 reductions, respectively, compared to the control (manure with untreated inoculum). They suggest that acidification treatment effects could be long-term by lowering the inoculation effect of residual acidified manure resulting in a reduced frequency of acidification to every other tank emptying. These laboratory results are promising, however there is need to evaluate inoculum acidification on a large scale in outdoor manure storage tanks.

The objectives of this research were to: a) quantify the effect of acidified aged manure as inoculum on CH_4, NH_3, and N_2O emissions from dairy manure storages and b) quantify changes in methanogen and bacterial abundance relative to CH_4 reductions.

6.2. Methods

6.2.1. Meso-scale chambers

The study was conducted at the Bio-Environmental Engineering Centre (BEEC) at Dalhousie University's Agricultural Campus in Truro, Nova Scotia. The research site contained 6 in-

ground, cement, meso-scale manure tanks (6.6 m^2 and 1.8 m deep). This site has been previously described by Wood et al. (2012) and Le Riche et al. (2016). Each tank was filled with 10.6 m^3 (160 cm depth) of liquid dairy manure, consisting of 20% inoculum (2.1 m^3) and 80% fresh manure (FM; 8.5 m^3). Manure was obtained from a local diary operation which housed 95 lactating cows in a free stall barn. The manure was gathered from an in-ground manure tank adjacent to the dairy barn and was a mixture of feces, urine, and sand bedding.

Two types of inoculum were used in this study: (i) 1-yr-old untreated manure, and (ii) 1-yr-old manure that was previously acidified (Table 6.1). This manure inoculum was obtained from the same farm in spring 2017 (12 months prior to the start of this trial). The previously acidified (PA) manure was acidified using sulphuric acid (70% H_2SO_4; 2.4 L m^{-3} manure) to pH 6. Both the PA manure and untreated manure inoculum remained in storage for 1-yr (Sokolov et al. 2019b).

The 6 manure tanks were assigned within 2 blocks, each containing 2 treatments and a control. Inoculum was prepared on May 15-16, 2018 by pumping out of old storages and distributing 2.1 m^3 to new storages using a pumping truck. The newly acidified (NA) inoculum treatment consisted 2.1 m^3 of 1-yr-old untreated inoculum and was acidified on May 17, 2018 with 1.1 L m^{-3} 70% H_2SO_4 (i.e. 12 L per 10.6 m^3 tank). The PA inoculum treatment consisted of 2.1 m^3 of 1-yr-old inoculum which had been acidified the previous year (spring 2017) at 2.4 L m^{-3} (i.e. 5.04 L added to 2.1 m^3; Table 6.1). Lastly, the control consisted of 2.1 m^3 of 1-yr-old untreated manure inoculum. Tanks were filled to full volume (10.6 m^3) with fresh manure on May 28 and 29, 2018 using a pumping truck to transport manure from the farm to the research site. The NA inoculum treatment received 1.1 L m^{-3} H_2SO_4, which is half as much as a previous meso-scale study (Sokolov et al. 2019b) but considerably more than in the laboratory study where rates were

only 0.16 L H_2SO_4 m^{-3} of total manure (i.e. 0.03 mL of 98% H_2SO_4 in 180 mL of stored manure; Sokolov et al. 2020).

Sulfuric acid (industrial grade) was obtained from Bebbington Industries (Dartmouth, NS) and was pumped into the inoculum manure using acid resistant tubing and a peristaltic pump. The tubing was attached to an aluminum pole which was moved around the inoculum as the acid was being pumped.

Table 6.1 Rate of 70% sulfuric acid (H_2SO_4) added to each treatment per year (L), where year 1 is prior to this study (2017) and year 2 is year of the study (2018).

		Control	PA	NA
		Acid addition L		
H_2SO_4	Year 1	0	25	12
	Year 2	0	0	12
	Total	0	25	24

6.2.2. Flux monitoring

Emissions of CH_4 and N_2O were monitored continuously from Jun 8 to Nov 10, 2018 (155 d). Each manure storage tank was covered by a flow-through, steady-state chamber (~13 m^3 headspace) consisting of an aluminum frame and 0.15 mm greenhouse plastic. Air was pulled through the chamber through intake slits at the front of the chamber and out through an exhaust fan and outflow exhaust duct and the opposite end. The rate of air flow within each chamber was approximately two full air exchanges per minute (~0.5 m^3 s^{-1}). The airspeed was measured within each exhaust duct using cup anemometers recorded by a CR1000 data logger (Campbell Scientific, Edmonton, AB). The air temperature within each chamber was measured using copper-constantan thermocouples at 10 cm above the manure surface and along with manure temperature at 80 cm depth and 150 cm depth, recorded by the same CR1000 data logger

(Campbell Scientific, Edmonton, AB). Ambient air temperature was obtained from the nearest Environment Canada climate station (Debert, NS, 45.42 N, 63.42 W; Climate ID: 8201380).

6.2.3. Methane and Nitrous Oxide

Air samples were continuously pumped (RC0021, Busch Vacuum Pumps and Systems, Boisbriand, QC) from the exhaust duct of each tank and two ambient inflow locations, and carried through polyethylene tubing (3.2 mm i.d.; Rubberline Products Ltd., Kitchener, ON) to a 8×2 manifold (Campbell Scientific In., Logan, UT) containing 12 V DC valves (The Lee Co., Essex, CT). The valves directed two samples every 30 sec through high-flow air dryers (Perma Pure LLC.; Toms River, NJ) and into one of two tunable diode trace gas analyzers (TDLTGA, Campbell Scientific, Logan, UT). Sample CH_4 and N_2O concentrations were continuously recorded by a CR5000 data logger (Campbell Scientific Inc., Logan, UT) and an adjacent PC computer monitored the analyzer performance by running the TDLTGA software (Campbell Scientific, Logan, UT).

Concentrations were averaged hourly and used to calculate flux rates using to the following:

$$\boldsymbol{F = \frac{Q}{A}(C_o - C_i)} \qquad \text{Eq. 6.1}$$

where F is the hourly flux (mg m^{-2} h^{-1}), Q is the flowrate of air out of the chamber (m^3 h^{-1}; calculated using average hourly windspeed × cross-sectional area of the exhaust duct (0.0645 m^2)), A is the surface area of the manure surface (6.63 m^2), and C is the concentration of gas (mg m^{-3}) in the ambient inflow air (C_i) and sample outlet air (C_o).

Due to technical issues, block 1 had missing flux data Aug 21-Sep 2, and block 2 had missing data Jul 18-Sep 2. This resulted in missing the peak fluxes in block 2 tanks. Linear interpolation

was used to estimate the missing data, although the values were likely underestimated. All values are presented as treatment average.

6.2.4. Ammonia

Ammonia concentrations were determined using 125 mL 0.005 M H_3PO_4 acid traps. Three times per week, air was pumped (Model 2107CA20B; Thomas Pumps and Compressors, Sheboygan, WI) from the exhaust of each tank and two ambient inflow locations and bubbled through acid traps (dispersion tubes id = 35 mm) at 1.5 L min^{-1}. Air was continually pumped through the traps for 24 h at each deployment. Airflow for each sample was measured using inline flow meters (Gallus 2000; Actaris Metering Systems, Greenwood, SC). Following deployment, evaporated liquid was replaced to 125 mL and a sample frozen until analysis. Samples were shipped to Agriculture and Agri-Food Canada (Ottawa, ON) where they were analyzed for NH_3-N using the QuikChem® Method 12-107-06-2-A modified for 0.005 mol L^{-1} H_3PO_4 matrix using a Lachat QuikChem FIA+ Q8500 Series. Daily gas concentrations were calculated using the following:

$$C_{NH_3\ air} = \frac{C_{NH_3\ aq} \times V_{aq}}{V_{air}} \qquad \text{Eq. 6.2}$$

where $C_{NH_3\ air}$ is the daily NH_3-N concentration (mg m^{-3}), $C_{NH_3\ aq}$ is the NH_3-N concentration in sample liquid (mg L^{-1}), V_{aq} is the volume of liquid in the acid trap (L), and V_{air} is the volume of air pumped through the acid (m^3) (Hofer 2003).

Ammonia emissions on days that were not sampled were estimated using linear interpolation and daily total NH_3-N losses were added together to find the entire monitoring period.

6.2.5. Manure sampling and analysis

Six FM composite samples were taken during tank filling (May 29). Manure in each tank was sampled monthly throughout the study with one sample per tank made from a composite of 12

subsamples. Subsamples were taken from each tank in a grid at two depths and 6 locations. All samples were kept frozen until analyzed at the Nova Scotia Department of Agriculture's Provincial Soils Lab (Bible Hill, NS). Samples were analyzed for total solids (TS) and volatile solids (VS) (American Public Health Association method 2540 B), total nitrogen (TN) (combustion method AOAC 990.03-2002), ammonium-N (TAN) (American Public Health Association method 4500-NH_3 B), and pH using an electrode (American Public Health Association method 4500-H^+) (Clesceri et al. 1998). To verify pH, a FieldScout pH 400 meter (Spectrum Technologies, Aurora, IL, USA) was used to measure pH in the manure at 10, 50, 100, and 150 cm across 6 locations in each tank (24 pH points) on May 26, Jul 1, and Jul 31, 2018. These are not reported in the paper but verify the results of lab analysis.

For microbial analysis, duplicate composite samples were taken during storage tank filling (May 29, 2018) of FM, untreated inoculum, and previously acidified inoculum. Throughout the study, monthly composite manure samples were collected in duplicate and kept frozen until nucleic acid extraction. For each sampling, ~2 g of manure sample was stored in 5 mL of LifeGuard soil preservation solution (MoBio Laboratories Inc., Carlsbad, CA).

6.2.6. Nucleic acid extraction and quantitative real-time PCR

Based on the typical CH_4 emission curve, three sampling dates and starting FM and the two inoculums were chosen for analysis. The DNA or RNA PowerSoil total DNA/RNA isolation kit with RNA/DNA elution accessories (MoBio Laboratories, Inc., Carlsbad, CA) were used for DNA or RNA extraction. In triplicate, 8 µL of each extracted RNA sample was reverse transcribed to cDNA using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA) following manufacturer's protocols.

Real-time qPCR was performed using an Applied Biosystems StepOnePlus real-time PCR system using clear 96-well PCR plates (Bio-Rad Laboratories, Inc, Hercules, CA). The total and active fraction of the methanogen populations were quantified by targeting methyl coenzyme A reductase (*mcrA*) genes and transcripts, respectively, using mlas-mod F and mcrA-rev-mod R primers (Habtewold et al. 2018). Bac 338F and Bac 518R primer sets were used to quantify bacterial populations by targeting 16S rRNA genes and transcripts (Habtewold et al. 2018). Each reaction well contained 10 μL of Ssofast EvaGreen supermix (Bio-Rad Laboratories, Inc.), 1 μL (10 pM) of each primer, 2 μL of DNA or cDNA, and 6 μL of PCR-grade water. Plasmid standard curves were prepared for *mcr*A from *Methanosarcina mazei* (ATCC 43340), and for 16S rRNA genes, plasmid with 16S rRNA gene insert from soil bacterium *Clostridium thermocellum* was used. PCR thermal cycling parameters were as described by Habtewold et al (2018). The *mcr*A gene standard curve had an efficiency of 101.6%, r^2 of 0.99, and slope of -3.29. The highest diluted standard had a cycle of quantification of 30.2 and no-template controls of 31.4. The 16S rRNA gene standard curve had an efficiency of 100.1%, r^2 of 0.998, and slope of -3.32. The highest diluted standard had a cycle of quantification of 27.0 and no-template controls of 28.9. StepOne software v2.3 (Bio-Rad Laboratories, Inc, Hercules, CA) was used to calculate sample copy numbers.

6.2.7. Data analysis

To compare treatments based on their global warming potential, GHG emissions were converted to 100-yr CO_2-equivalent (CO_2-eq) values and summed. Conversion values for the global warming potentials of CH_4 and N_2O were 34 and 298, respectively (IPCC 2014). The contribution of indirect N_2O emissions from NH_3 volatilization were calculated using the IPCC emission factor of 0.01 (Dong et al. 2006).

Given that PA inoculum could reduce the need for acidification following every other emptying event, to compare use of PA inoculum and NA inoculum it is necessary to compare estimated total emissions over two storage periods. The total PA inoculum over 2 storage periods was calculated using the following:

$$\boldsymbol{Total\ CH_4 = Acidified\ manure\ CH_4 + PA\ inoculum\ CH_4} \quad \text{Eq. 6.3}$$

where Total CH_4 is the total production over 2 storage periods, Acidified manure CH_4 is the production from one storage period where all manure was acidified (reported by Sokolov et al. 2019), and PA inoculum CH_4 is the total CH_4 production from the PA inoculum treatment.

The NA inoculum for 2 storage periods was calculated by doubling the total CH_4 production from the NA inoculum treatment. Lastly, the control for 2 storage periods was calculated by doubling the total CH_4 production from the controls. Note that this assumes both storage periods to have the same temperatures. Therefore, the two storage periods do not represent spring/summer and fall/winter, as emissions would be dramatically different during cold weather storage.

For each treatment the methane conversion factors (MCF) was calculated following IPCC methods (Dong et al. 2006). The calculation used the average VS of FM (disregarding VS of the inoculum) and maximum potential CH_4 production (B_o) of 0.24 m^3 CH_4 kg^{-1} VS. Cumulative N_2O and NH_3-N emissions for each tank were scaled by TN and TAN in FM and then averaged for each treatment.

Treatment effects were assessed using repeated measures, mixed linear model analysis using PROC Mixed in SAS software (SAS Institute Inc., Cary, NC) using the Kenward-Roger fixed effects method on total biweekly CH_4, N_2O, and NH_3 emissions. The CH_4 data was skewed and

therefore log transformed to conform to normality. The spatial Gaussian covariance structure was chosen based on best fit statistics. Significance was considered when $p<0.05$. Treatment effects on *mcrA* and 16S rRNA gene and transcript copy numbers over all dates were assessed using a general linear model using PROC GLM in SAS software, which uses ordinary least squares with Sidak adjustment to control familywise error. Effect size was calculated using partial eta^2 (η_p^2). Significant results were followed up with a post hoc Sidak groupings comparison using a significance of $p<0.05$.

6.3. Results

6.3.1. Manure characteristics

Average ambient air temperature during the study (Jun 1 – Oct 31, 2018; 160 d) was 15.1°C as recorded by the closest Environment Canada climate station. The 30-yr normal for this location, Jun – Oct, is 14.7±0.2°C (Mean±SD). The temperature inside the tank chambers (10 cm above the manure surface) was 17.6±0.8°C, which was, on average, 2.6°C warmer than the ambient air (Figure 6.1). The average manure temperature in the tanks was 13.7±0.1°C at 150 cm depth and 17.6±0.1°C at 80 cm depth. The manure temperature peaked at week 12 (d 68, Aug 14) at 80 cm (20.9°C) and week 15 (d 91, Sep 6) at 150 cm (15.6°C). The CH_4 production followed a similar pattern, peaking a week earlier (d 61, Aug 7). Following the peak, the 80 cm temperature quickly fell. By the end of the study the temperature at 80 and 150 cm were both on average 14.3°C (~ 122 d, Oct 7).

The manure pH was not as expected, with no clear treatment differences until d 85 into the study (Figure 6.1). The control was expected to have the highest pH, but was on average the lowest on Jun 15, two weeks after the start of the study. The pH dropped throughout storage until Sept

when it increased. By Sept 8, the control tanks had the highest pH and the NA inoculum tanks had the lowest. This trend continued in Oct as well (Figure 6.1).

The TS, VS, and N were all highest in the FM and fell markedly by Jun 15 (Table 6.2). This is most likely due to settling of solids which occurs rapidly following storage tank filling, and issues with unrepresentative sampling of the manure depth (Sokolov et al. 2019b). The control had consistently the least VS, TS, and N (Table 6.2). This may be due to faster degradation of organic matter and loss of N to the atmosphere, although given the small differences, it may also be due to natural variability in manure (Sokolov et al. 2019b).

Table 6.2 Manure dry matter (%), volatile solids (%), total nitrogen (%), and ammonium-nitrogen (%) sampled from fresh manure (FM), one composite sample during tank filling (May 29), and stored manure from each tanks on days 7 (June 15), and 145 (October 31, 2018).

		Control	**PA-Inoc**	**NA-Inoc**
Dry Matter (%)	FM	17.60	17.60	17.60
	15-Jun	12.45	12.69	11.92
	31-Oct	14.24	14.89	14.44
Volatile Solids (%) dry basis	FM	8.06	8.06	8.06
	15-Jun	6.02	6.10	5.99
	31-Oct	5.59	5.99	6.40
Nitrogen (%) dry basis	FM	0.39	0.39	0.39
	15-Jun	0.26	0.26	0.30
	31-Oct	0.27	0.30	0.29
Ammonium-N (%) dry basis	FM	0.16	0.16	0.16
	15-Jun	0.05	0.06	0.09
	31-Oct	0.09	0.10	0.10

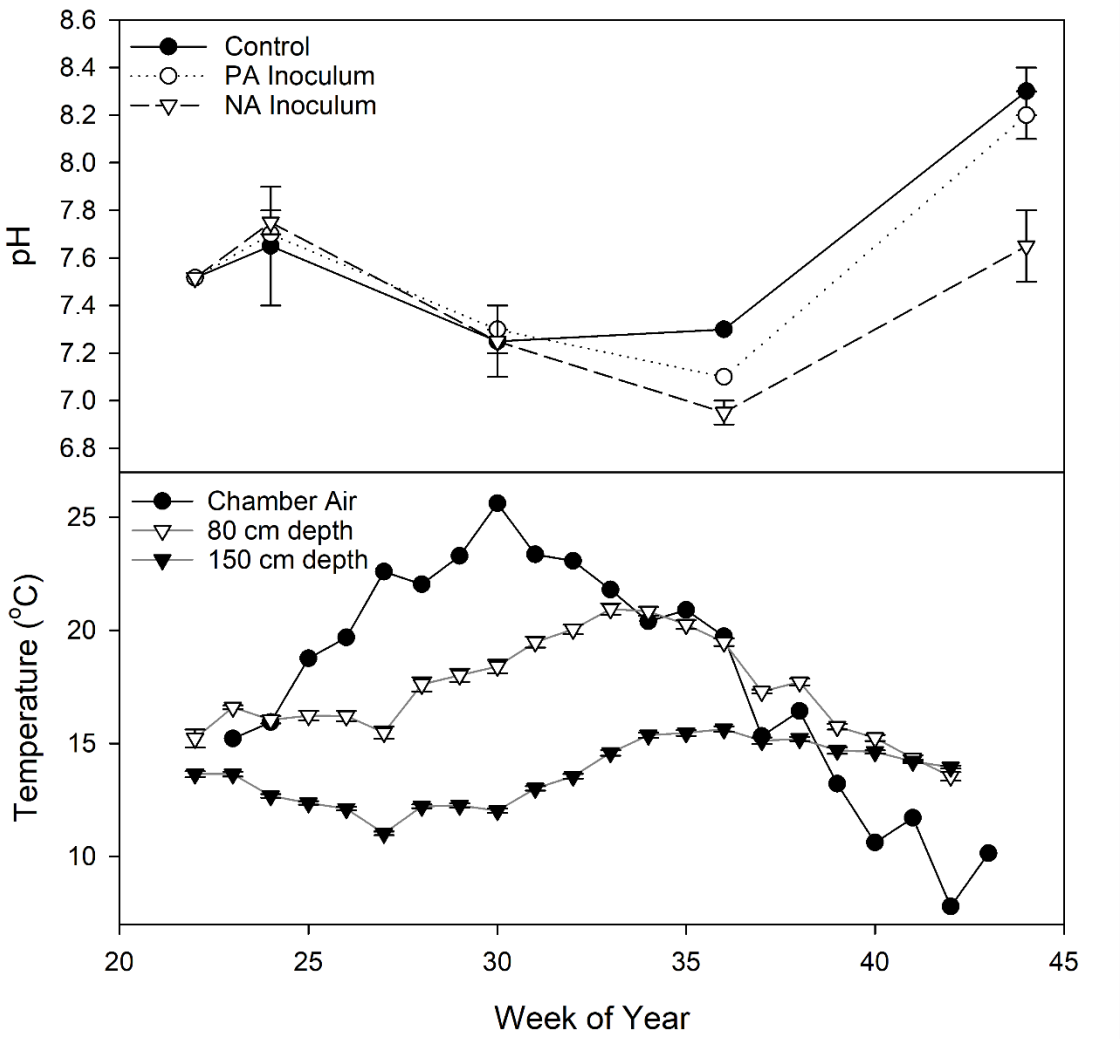

Figure 6.1 Manure pH (top) in control, previously acidified (PA) inoculum, and newly acidified (NA) inoculum treatments, samples from on May 29 from fresh manure and stored manure on June 15, July 27, September 8, and October 2018 (days 7, 49, 92, and 145). Weekly average temperature (bottom) averaged across all tanks, of chamber air 10 cm above manure and of manure at 80 cm and 150 cm depth.

6.3.2. Greenhouse Gas Emissions

6.3.2.1. Methane

Results of the mixed linear model show a significant CH_4 fixed effect due to treatment ($p<.0001$), time ($p<.0001$), and a combined effect of treatment and time ($p<.0001$). The average CH_4 emissions were 36.1, 22.3, and 8.2 g m^{-2} d^{-1} from the control (80% FM and 20% inoculum), PA inoculum (80% FM and 20% previously acidified inoculum), and NA (80% FM and 20% acidified inoculum) storages, respectively (Table 6.3). All treatments had similar lag phases of ~40 d, although even during this time the control produced 31% more CH_4 than the NA

inoculum tanks and 27% more than the PA inoculum tanks (Figure 6.2). The rate of growth following the lag was much higher in the control storage tanks. In fact, between day 40 (Jul 17) and day 110 (Sep 25) the largest treatment differences were recorded. At this time, NA inoculum tanks produced 82% less CH_4, while the PA inoculum tanks produced 47% less CH_4 compared to the control. After 110 d, fluxes were similar to the control and PA inoculum tanks (<25% difference). The NA inoculum tanks continued to produce less (56-80%) CH_4 than the control throughout the end of the study.

Table 6.3 Total (g m^{-2}, kg) and daily mean (g m^{-2} d^{-1}) methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3) for manure with untreated inoculum (control), previously acidified inoculum (PA-Inoc), and newly acidified inoculum (NA-Inoc) in each block for the entire study period Jun 8 – Oct 31, 2019 (145 d).

CH_4	**Control**	**PA Inoculum**	**NA Inoculum**
Daily mean g m^{-2} d^{-1}	36.1±27.7	22.3±17.8	8.19±5.64
g m^{-2}	5266	3258	1196
g m^{-3}	3291	2036	748
kg	34.9	21.6	7.9
m^3	53.2	32.9	12.1
VS, kg	671	679	667
Potential B_0 × VS	161	163	160
MCF	0.33	0.20	0.08
N_2O			
Daily mean mg m^{-2} d^{-1}	76.4±65.1	38.4±29.9	22.3±23.7
g m^{-2}	11.2	5.61	3.00
g m^{-3}	6.97	3.51	1.88
g kg^{-1} TAN	13.3	5.93	2.23
g kg^{-1} TN	2.60	1.28	0.62
NH_3			
Daily mean g m^{-2} d^{-1}	4.55±2.28	3.55±2.00	3.19±1.52
g m^{-2}	482	370	325
g m^{-3}	301	231	203
g kg^{-1} TAN	574	370	226
g kg^{-1} TN	113	84.7	65.7

The total CH_4 production was 5.27, 3.26, and 1.20 kg m^{-2} from control, PA inoculum, and NA inoculum tanks, respectively (Table 6.3). The PA inoculum (38%; $p<.0001$) and NA inoculum (77%; $p<.0001$) treatments produced significantly less CH_4 compared to the control treatment. The NA inoculum treatment produced significantly less CH_4 (63%; $p<.0001$) than the PA inoculum. These treatment differences were the same on a VS basis.

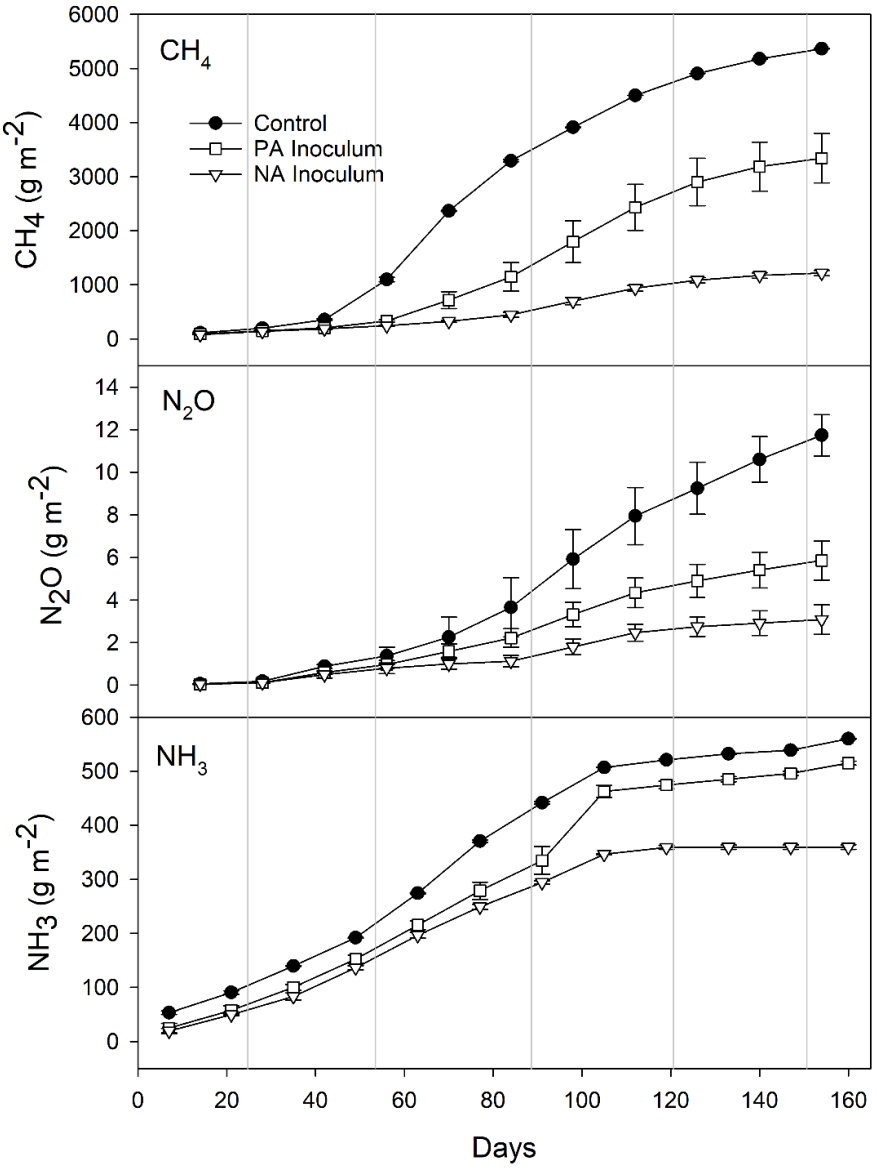

Figure 6.2 Average cumulative methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3) emissions summed every 14 d from manure with untreated inoculum (control), manure with previously acidified (PA) inoculum, and manure with newly acidified (NA) inoculum for the entire study period (Jun 8 – Oct

31, 2019; 145 d). Error bars show standard deviation and vertical grey lines denote the end of a month, starting with June and ending with November.

6.3.2.2. Nitrous Oxide

Results of the mixed linear model show a significant N_2O fixed effect due to treatment ($p<.0001$), time ($p<.0001$), and a combined effect of treatment and time ($p=0.0141$). The daily average N_2O emissions were 76.4, 38.4, and 22.3 mg m^{-2} d^{-1} from control, PA inoculum, and NA inoculum, respectively (Table 6.3). After interpolation, the total N_2O production was 11.2, 5.6, and 3.0 g m^{-2} from control, PA inoculum, and NA inoculum, respectively (Figure 6.2). This represented a significant ($p=0.0015$) 50% reduction using PA inoculum and a significant ($p<.0001$) 73% reduction using NA inoculum, compared to the control. The NA inoculum produced 47% as much N_2O than the PA inoculum tanks ($p=0.1091$). The treatment differences increased slightly (<10%) when scaled by TAN and TN in the manure.

6.3.2.3. Ammonia

Results of the mixed linear model show a significant NH_3 fixed effect due to treatment ($p<.0001$), time ($p<.0001$), and a combined effect of treatment and time ($p=0.0351$). The average NH_3 emissions were 3.53, 3.28, and 2.76 g m^{-2} d^{-1} from control, PA inoculum, and NA inoculum, respectively (Table 6.3). The total NH_3 emissions over the entire study were 540, 502, and 382 g m^{-2} from control, PA inoculum, and NA inoculum, respectively (Figure 6.2). This represented a significant ($p=.0001$) 7% reduction using PA inoculum and a significant ($p<.0001$) 29% reduction using NA inoculum, compared to the control. The difference in NH_3 volatilization between PA and NA inoculum were 25% ($p=0.1326$), which is likely due to the similar manure pH.

6.3.3. CO_2–Equivalent Emissions

On a CO_2-eq basis, the total GHGs were 94-97% comprised of CH_4 emissions, due to the anaerobic conditions within the manure storages (Table 6.4). Clear treatment difference was

observed, where PA inoculum reduced total GHGs by 38% and NA inoculum reduced total GHGs by 77%, compared to control. All sources of GHG were reduced due to PA and NA inoculum, although CH_4 was the most important in reducing total GHGs.

Table 6.4 Total greenhouse gas emissions and the contributions of methane (CH_4), direct nitrous oxide (N_2O), and indirect N_2O due to ammonia NH_3 volatilization, and estimated 1-yr and 2-yr total on a CO_2-equivalent basis, and from the control, previously acidified inoculum treatment (PA), and newly acidified inoculum treatment (NA). Year 2 represents emissions from this trial, while year 1 represents emissions from a theoretical previous trial, where the control the PA had new acidification, while the control and NA were the same for both years.

		Control	PA	NA
		CO_2-equivalent kg m^2		
CH_4	Year 1	179	19.7	40.7
	Year 2	179	111	40.7
	Total	358	130	81.4
N_2O-direct	Year 1	3.32	1.39	0.89
	Year 2	3.32	1.67	0.89
	Total	6.64	3.06	1.78
N_2O-indirect	Year 1	2.26	1.06	1.52
	Year 2	2.26	1.73	1.52
	Total	4.52	2.79	3.04
Total over 1 yr		185	22	43.1
Total over 2 yrs		369	136	86.2

6.3.4. Methanogens and bacteria

The results of the 2-way ANOVA on copies of *mcrA*, *mcrA* transcript, 16S rRNA, and 16S rRNA transcript are shown in Table 6.5. There were significant treatment and month effects on *mcrA* ($p<.0001$ for both) and 16S rRNA ($p<.0126$ and $p<.0043$, respectively) but not in *mcrA* or 16S rRNA transcript.

Table 6.5 Results of 2-way ANOVA, effects of treatment and month for copy number of mcrA, mcrA transcript, 16S, and 16S transcript (copies g^{-1} dry matter). Showing mean squared error (MSE), degrees of freedom (df), F-value (F), p-value (p), eta-squared (η_p^2), and 90% confidence intervals (CI_{90}).

Data	Source	MSE	df	F	*p*	η_p^2	CI_{90}	
mcrA	Treatment	4.58	2	50.5	<.0001	0.3908	0.5139	0.7388
	Month	4.40	2	48.5	<.0001	0.3757	0.5024	0.7318
mcrA transcript	Treatment	0.496	2	0.17	0.8423	0.0044	0.0000	0.0445
	Month	11.8	2	4.11	0.0229	0.1041	0.0111	0.2667
16S	Treatment	44.0	2	4.86	0.0126	0.1263	0.0222	0.3031
	Month	56.3	2	6.23	0.0043	0.1618	0.0434	0.3428
16S transcript	Treatment	0.145	2	1.03	0.364	0.0391	0.0000	0.1300
	Month	0.017	2	0.12	0.885	0.0046	0.0000	0.0318

The FM had higher copies of *mcrA* transcript and bacterial 16S rRNA genes and transcript per gram of dry manure than untreated inoculum (30 – 45%; percentages are calculated on values prior to log transformations) sampled prior to the start of the study (Table 6.5). An exception was *mcrA* gene in untreated, control inoculum which had 64% more copies per gram of dry manure of than FM. These results differed from Habtewold et al. (2018) who reported more (11-458%) copies of genes and transcripts of both *mcrA* and bacterial 16S in inoculum compared to FM. At the start of the trial, previously acidified inoculum had lower *mcrA* copies of genes and transcript (88%) and lower 16S rRNA genes and transcripts (90%) compared to the untreated inoculum. Given that both inoculums were stored for 1-yr under the same conditions, the difference in abundance are likely due to acidification with H_2SO_4 1-yr prior.

Table 6.6 Copies of mcrA and 16S gene and transcript from fresh manure (FM) and inoculum sampled on tank filling day (May 29, 2018), and from composite samples of stored manure on July 27, September 8, and October 31, 2018 from control, newly acidified inoculum (NA-Inoc), and previously acidified inoculum (PA-Inoc) treatments.

		Control	**PA-Inoc**	**NA-Inoc**
mcrA gene	FM	7.71±0.003	7.71±0.003	7.71±0.003
	Inoculum	8.16±0.005	7.13±0.011	8.16±0.005
	27-Jul	8.14±0.005	7.81±0.016	7.79±0.013
	8-Sep	8.46±0.008	8.27±0.009	8.02±0.011
	31-Oct	8.56±0.003	8.36±0.001	8.04±0.12
mcrA transcript	FM	6.73±0.14	6.73±0.14	6.73±0.14
	Inoculum	6.51±0.07	5.68±0.13	6.51±0.07
	27-Jul	8.16±0.12	7.31±0.05	6.83±0.03
	8-Sep	5.99±0.16	6.71±0.04	7.56±0.04
	31-Oct	6.73±0.01	7.29±0.05	6.78±0.06
16S gene	FM	11.5±0.02	11.5±0.02	11.5±0.02
	Inoculum	11.2±0.005	10.4±0.02	11.2±0.005
	27-Jul	10.9±0.02	10.8±0.04	10.8±0.02
	8-Sep	10.9±0.01	10.9±0.01	10.9±0.02
	31-Oct	10.9±0.02	10.9±0.04	10.7±0.08
16S transcript	FM	13.4±0.14	13.4±0.14	13.4±0.14
	Inoculum	13.2±0.15	12.1±6.98	13.2±0.15
	27-Jul	13.2±0.08	13.1±0.03	13.1±0.06
	8-Sep	10.5±0.04	11.6±3.82	13.1±3.66
	31-Oct	12.3±0.06	10.9±3.75	12.9±0.09

Averaged over the entire study period, the control had significantly more *mcrA* gene copies compared to PA inoculum (39%) and NA inoculum tanks (65%, $p<.05$;Table 6.5). The PA inoculum tanks had significantly more *mcrA* gene copies than NA inoculum tanks (43%, $p<.05$).

The *mcrA* transcript copies were variable over time, although the most marked difference between treatments was Jul 27 (d 42) when the NA inoculum and PA inoculum were 95% and 85% less than the control copies, respectively. This corresponds with the initial increase in CH_4

emissions. The average CH_4 emissions during the sampling week were 43.0, 7.70, and 4.12 g m^{-2} d^{-1} from control, NA inoculum, and PA inoculum treatments, respectively.

On Sept 8 (85 d) the NA inoculum treatment had the highest copies *mcrA* transcript, with the control and PA inoculum having 97% and 86% fewer copies, respectively (Table 6.5). This corresponds to CH_4 emissions during the sampling week of 43.4, 55.5, and 21.4 g m^{-2} d^{-1} from control, NA inoculum, and PA inoculum, respectively.

Lastly, on Oct 31 (108 d) the PA inoculum had the highest copies of *mcrA* transcript, with control and NA Inoculum having 81% and 86% fewer copies, respectively (Table 6.5). This corresponds to CH_4 emissions during the sampling week of 30.0, 34.2, and 11.0 g m^{-2} d^{-1} from control, NA inoculum, and PA inoculum, respectively.

The 16S rRNA gene copies varied less over time and between treatments (Table 6.5). The 16S rRNA transcript copies in the control treatment increased and decreased following the same pattern as the *mcrA* transcript copies. This pattern was not observed in the NA and PA inoculum, suggesting that the methanogen and bacterial communities had differing influences.

6.4. Discussion

Storages with NA inoculum reduced total GHGs by 77%, while PA inoculum reduced emissions by 38%, compared to the control. Sokolov et al. (2019b) acidified manure with no inoculum at rates of 1.4 and 2.4 L 70% H_2SO_4 m^{-3} and reported 85% and 88% reductions in total GHGs, respectively. Our results were slightly lower, which is likely due to the lower rate of acid and the presence of an inoculum. In a lab study, Sokolov et al. (2020) stored FM with previously acidified (2.4 L 70% H_2SO_4 m^{-3}; 6-month old) inoculum and newly acidified inoculum at (0.17 L 98% H_2SO_4) 17°C, 20°C, and 23°C and reported average CH_4 reductions of 82% and 63%,

respectively, across all temperatures. The PA inoculum in the lab study was more effective, with 82% reductions compared to the 38% reduction in this study. This difference may be due to the lab scale or age of the inoculum. The NA inoculum in the lab study had a much lower rate of H_2SO_4, (0.16 vs 0.79 L pure H_2SO_4 m^{-3} total manure) which explains the lower (63%) reduction of CH_4.

All contributing GHGs were reduced using NA and PA inoculum. This is important to note, as often mitigating practices reduce one GHG in exchange for increasing another. Although there were clear GHG reduction treatment differences, the pH did not have corresponding differences. This could be due to sulfide (derived from sulfuric acid) inhibiting methanogenesis, rather than pH change alone (Petersen et al. 2012). In fact, the pH was nearly identical among treatments until day 92, thereafter the acidification treatments showed lower pH. Sokolov et al (2019) also reported variable pH levels in storage tanks following acidification, although pH levels stabilized 35 d into the trial. Others have only observed increases in pH throughout storage due to natural processes re-establishing a neutral pH following acidification (Petersen et al. 2012; Shin et al. 2019). However, this might be due to better mixing of acid in initial short-term storage. Future research should examine the effects of methanogenesis inhibition by sulfide at different pH levels, corresponding hydrogen sulfide (H_2S) production, and resulting total GHG emission reduction from liquid dairy manure.

The NA inoculum treatments reduced total GHGs and total CH_4 by 77%. Both the control and the NA inoculum received the same untreated inoculum and FM, although NA inoculum received 1.2 L 70% H_2SO_4 m^{-3} (total manure in storage) into the inoculum prior to FM addition. This is similar to results from Sokolov et al. (2019) who reported an average 88% reduction of CH_4 from acidifying FM using 1.4-2.4 mL 70% H_2SO_4 L^{-1} manure. Results of real-time

qPCR suggest that the reduction is due to disruption in methanogen activity. On the sampling closest to peak emissions (Jul 27), the *mcrA* gene and transcript were lower in the NA inoculum tanks compared to the control. Habtewold et al (2018) also found disruption of methanogen communities through reduced *mcrA* gene and transcript following acidification.

The PA inoculum treatments reduced total GHG and total CH_4 by 38% using no acid in this storage period and only inoculum that was acidified 1-yr prior. Results of the real-time qPCR suggest that the reduction is due to reduced methanogen activity in the inoculum. The previously acidified inoculum had markedly lower *mcrA* gene and transcript compared to the untreated inoculum at the start of the trial. The same results are observed during the following sampling event on Jul 27, which was during the time of peak emissions (40 – 110 d). This suggests that the reduced methanogen activity, expressed as *mcrA* transcript, in the PA inoculum led to lower methanogen activity later in the storage. This was also suggested in Chapter 4, using PA inoculum had similar CH_4 production as FM with no inoculum in a laboratory incubation study (Sokolov et al. 2020). They reported similar CH_4 reductions of 49% using PA inoculum and 55% using no inoculum at 23°C. Ngwabie et al. (2016) similarly reported 36% reductions in CH_4 from manure with no inoculum compared to manure with 20% inoculum (163 d storage).

Given that PA inoculum can reduce the need for acidification to every other filling, it is important to compare estimated total GHG emissions from PA inoculum and NA inoculum over two storage periods. Acidifying all manure in the first storage period and using the PA inoculum in the second period reduced an estimated total GHG emissions by 62%, compared to the control. Using NA inoculum over 2 storage periods reduced total GHG emissions by 77%, compared to the control. The amount of acid using PA inoculum compared to NA inoculum was nearly identical in both treatments (1.1 vs 1.2 L m^{-3} yr^{-1}), although acidifying once accompanied

a 38% decrease in GHG. Given that the cost of acid would be nearly the same, the best management practice would be to acidify each year. However, other factors are important to consider, such as the cost of the acidification process (acid delivery, equipment rental, labour, etc.) which is currently unclear and may be prohibitive to farmers. Additionally, removal of manure in the fall with PA inoculum accompanying winter storage may not reduce emissions further, as winter conditions cause very low GHGs regardless of inoculum and acid presence. However, spring emptying with PA inoculum accompanying summer storage could reduce the frequency of acidification and reduce GHG emissions by 62%.

6.5. Conclusion

Acidification of manure inoculum (1.1 L 70% H_2SO_4 m^{-3} total manure in storage) reduced overall GHG emissions by 77% compared to fresh manure and untreated inoculum. Using previously acidified inoculum reduced overall GHG emissions by 38% and by 62% when considered over 2 yr.

The largest contributing GHG was CH_4, which was reduced by 77% using newly acidified inoculum and 38% using previously acidified inoculum. Significant treatment effects on *mcrA* and 16S rRNA suggest that this reduction was due to disruption of methanogen activity. Emissions of N_2O were reduced by 73% using NA inoculum and 50% using PA inoculum, while NH_3 was reduced by 33% and 23%, respectively. Over 2 storage periods, the amount of H_2SO_4 was nearly identical between PA and NA inoculum treatments, however PA inoculum allowed for biennial acidification while still retaining good GHG reductions due to reduced inoculating effects. This may allow farmers to reduce expenses associated with acidification while still mitigating GHGs, although more research is needed to validate its applicability at the farm scale.

7. Greenhouse gases from manure storage: A decade of research

7.1. Introduction

Liquid dairy manure is increasingly preferred on dairy farms, as it offers ease of transport and disposal for animal waste, wash water, and bedding material (VanderZaag et al. 2010b). Prior to spreading onto fields, however, liquid manure is stored in tanks which are usually emptied once or twice a year. This creates a point source of greenhouse gas (GHG) emissions to the atmosphere. The most prominent gases include methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3). CH_4 and N_2O are GHGs which contribute to global warming at a rate of 25 and 298 times that of carbon dioxide, while NH_3 is a toxic gas which is hazardous to human health and contributes to environmental acidification (IPCC 2014).

Manure is a unique environment, which is mostly liquid (>75% water) and yet high in organically degradable compounds (Agriculture and Agri-Food Canada 1980). During animal digestion, 60-90% of nutrients (potassium, nitrogen, and phosphorus) from ingested feed are excreted (Agriculture and Agri-Food Canada 1980). The organic matter (OM) in manure, which is partially digested and in various forms of decomposition, undergoes further degradation processes during storage to produce CH_4, N_2O, and NH_3. Production of GHGs can be studied at different scales, from microscopic – looking at microbial colonies, to whole farm – looking at emissions with a systems approach. Different scales of research will give different information, interpretations, as well as extrapolation power. When testing mitigation practices, monitoring on-farm manure storages provides real-scale results, although lack replication and manipulation power. A good alternative to on-farm research are meso-scale studies, which contain multiple, experimentally sized manure storages. They are functionally small manure storages, which allow for environmental conditions similar to larger tanks (e.g. crust formation, temperature depth

gradient, etc). However, they also allow for replications, manipulation, and direct monitoring of GHG emissions and environmental conditions.

Since 2006, studies at the Dalhousie University Bio-Environmental Engineering Centre (BEEC) have addressed gaseous emissions from stored liquid dairy manure in meso-scale manure tanks. The purpose of these studies has been to identify emission mitigation strategies and inform best manure management practices. Treatments studied include: natural and synthetic covers, reducing volatile solids (VS) through dilution or changing bedding type, inoculum removal, and acidification.

Early research at BEEC 2006 – 2008 focused on use of natural and synthetic covers. These covers block or slow the movement of gases, forcing CH_4 oxidation and N_2O reduction to N, therefore possibly reducing GHG emissions (Alexander 1977; VanderZaag et al. 2009, 2010b; Owen and Silver 2015). In 2010, 2013, and 2014 studies focused on effects of in-barn manure management and its effect on emissions (Wood et al. 2012; Le Riche et al. 2016, 2017). Specifically, the effect of VS and TS concentration on GHG emissions. The availability of VS directly reduces substrates for microorganisms which produce biogas, while TS may change the movement of gases in manure. From 2011 – 2016 four trials explored complete versus partial cleaning of tanks with the intent of removing inoculating microbial colonies (Wood et al. 2014a; Ngwabie et al. 2016; Sokolov et al. 2019a; Le Riche et al. 2020). Inoculum remaining in manure tanks increases the biogas production and reduced the length of the initial production lag (Jayasundara et al. 2016; Ngwabie et al. 2016). Latest research at BEEC 2017 – 2018 has focused on manure acidification with sulphuric acid (H_2SO_4) (Habtewold et al. 2018a; Sokolov et al. 2020). Acidification reduces the pH below the preferred threshold for microbial growth.

This reduces biogas production by reducing microbial activity. Additionally, H_2SO_4 may have an effect on disrupting methane production due to sulphur interactions (Petersen et al. 2012).

In addition to mitigation strategies, studies from 2006, 2007, and 2016 studied experimental storage procedures at the meso-scale compared to on-farm. Agitation was added to the end of 2006 and 2007 trials, to quantify increased emissions on an event or overall trial basis (VanderZaag et al. 2009, 2010b). Agitation simulated the disruption of manure which occurs during pumping for field spreading. It allowed trapped bubbles to move upward and therefore create an GHG surge event. Experimental procedures generally end prior to tank emptying, therefore these emissions would not be measured. In 2016, gradual filling was compared to batch filling. This simulated on-farm tank filling, which occurs in daily or weekly events, as opposed to experimental procedures that involve batch filling once over the entire storage period (Sokolov et al. 2019a).

Lastly, of note is the trial from 2010 which included a parallel study assessing the best time of day for one-time flux sampling (Wood et al. 2013). This was done by looking at temporal CH_4 and N_2O data and finding the time of day which best represented the daytime mean emissions. The results of this study inform protocols for daily CH_4 and N_2O gas sampling.

This study combines the data from all 12 studies since 2006, reviewing the main results, and doing new analysis to uncover what can be learned by assessing the results collectively. The objectives are to determine emissions trends, best treatment effects, and make recommendations for practices that best mitigate emissions from stored liquid dairy manure.

7.2. Methods

7.2.1. Site Description

The Bio-Environmental Engineering Centre was constructed in 2005 at the at the Dalhousie University Agricultural Campus in Truro, NS. Six in-ground storage tanks were dug ~2 m deep into the ground and lined with concrete walls and floor. The total volume became 12 m^3 with a depth of 1.8 m and 6.6 m^2 surface area (1.73 m × 3.8 m). Tanks were built approximately 1 m away from each other in a row to receive similar weather conditions. Each tank was covered with a 1.5 m high steady-state chamber with a slanted roof, built from 0.15 mm greenhouse plastic stretched out across an aluminium frame. The CH_4 and N_2O monitoring equipment was housed in a small trailer parked perpendicular to the storage tanks about 10 m from the inflow. The NH_3 monitoring equipment was set up in a shed located near the outflow side of the tanks. Wiring and air tubing were protected from the elements using strong PVC and aluminium pipes.

7.2.2. Flow-through steady-state chambers

Steady-state chambers are used for continuous monitoring of an emitting substance. Steady-state conditions were achieved through continuous flow through of air, which prevents a build-up of emitted gases in the chamber. Continuous measurement of gas concentration for a known amount of air volume and air speed, ensured an accurate quantification of total gas produced. This method also allows for close monitoring of emission changes over small and large time periods.

Emissions of CH_4 and N_2O were measured using two Model 100A tunable diode laser trace gas analyzers (Campbell Scientific Inc., Logan, UT). The continuous data from the trace gas analyzers was recorded by a CR5000 datalogger (Campbell Scientific Inc., Logan UT) and an adjacent PC computer monitored the analyzer performance, pressure, temperature, and adsorption by running the Campbell Scientific software.

The NH_3 was sampled 3 × per week using a separate set of 25 m polyethylene tubing to pump air from each tank outflow and ambient location to a series of 125 mL of 0.005 mol L^{-1} phosphoric acid ammonia traps. The NH_3-N concentration in the sample solution was analyzed at a laboratory.

7.2.3. Environment and Manure

For all studies, fresh manure was sampled at the start of the trial and a composite from each storage tank end of the trial. Generally, the samples were analyzed for total solids (TS) and volatile solids (VS), total nitrogen (TN), ammonium-N (TAN), and pH. Average temperature was monitored at 1 or 2 depths in manure and 10 cm above the manure surface. These were recorded every minute and used to calculate hourly, daily, and whole study temperature averages.

For manures which produced crusts of >2cm, the thickness was measured using a wing-tipped rod. The rod was inserted through the manure crust and rotated so the wings would catch on the bottom of the crust when the rod was lifted. The crust thickness was measured as the distance from the crust surface to the top of the wings.

Due to the chamber covering the tank, evaporative water loss occurred throughout the monitoring periods, however rainfall was blocked. Therefore, rainfall was simulated using oscillating sprinklers and volume applied measured using a flow meter.

7.2.4. Changes in methods over time

Since 2006 the core methods for measuring CH_4, N_2O, and NH_3 changed little, although some changes were implemented as necessary. The same trace gas analyzers were used throughout all trials, but small changes were frequent due to necessary upkeep of equipment and replacement of parts due to wear and tear. Measuring airspeed in each storage outflow was upgrade in 2011

from discrete hotwire anemometer measurements to continuous cup anemometer measurements. Additionally, in 2006 – 2008 a CO_2 analyzer was set-up and monitored continuous CO_2 emissions. Due to issues with moisture condensation, the use of this analyzer was discontinued. The CO_2 emissions are not discussed in this study, although future works intends to re-establish CO_2 monitoring.

Trials all had different monitoring lengths and starting dates. Most trials started April – May, although 3 trials were over winter starting December – January. Winter data is reported, but not included in analysis. Winter storage was not considered a treatment. The manure volume also varied between trials, ranging from 8.6 – 11.4 m^3. Additionally, due to evaporation the volume of manure declined throughout trials. In 2006-2009 there was no water addition, resulting in evaporative losses, however, in 2010, sprinklers were installed in each storage and water was added based on weekly average rainfall calculated from the Environment Canada 30-yr normal weather data. Starting 2017 the amount of water added was based on evaporative loss to keep the volume of manure constant.

Manure temperature from mid-depth was missing in 2007, 2010, and 2011, and all manure temperature was missing from 2015 and 2014. The temperature used in this study is only from the mid-depth. Air temperature was missing from 2006 and 2015 trials. Lastly, manure sampling procedures changed somewhat over time. In 2016 composite manure sampling was made more rigorous by adding extra sub-samples from more depths and area locations. In 2006 and 2007 manure samples were not analyzed for VS, but instead for total carbon, and in 2012 manure was not analyzed for TAN, but for total Kjeldahl nitrogen.

7.2.5. Manure sources

Predominantly two farms sourced manure for the trials, Farm A and B (Table 7.1). Farm A was used from 2006 – 2014 and was part of the Dalhousie University Experimental Farm housing 40 milking cows in a tie-stall barn. The animal waste was mixed with water and sawdust bedding. Farm B was used from 2013 – 2018 and was a local commercial dairy farm housing 95 lactating cows in a free stall barn. The animal waste was mixed with water and sand bedding. Farms C, D, E, and F were used only for one study, Le Riche et al (2016), and all has manure mixed with sawdust bedding.

7.2.6. Studies and Treatments

Since its construction there have been 13 experiments performed at the experimental site. Most studies started with tanks being filled with fresh manure in the spring (April – June) and were continuously monitored until the trial end in the fall (October – December). There were 3 exception which started in December – January and finished in April – June (Table 7.1). Table 7.1 shows the citation and treatment for each trial. The main treatments that had been tested throughout the 10 years were: agitation, covers, total and volatile solid amounts, inoculum amount, and acidification.

7.2.7. Data Analysis

Flux data from each trial was obtained from each first author as daily averages (g m^{-2} d^{-1} or mg m^{-2} d^{-1}), along with manure and air temperature (°C), and manure characteristics (%TS, VS, TAN, TN, and pH). Where applicable, linear interpolation was used to fill in missing dates for CH_4, N_2O, and NH_3 daily emissions. Emission data was predominantly left in units of g m^{-2} units for simplicity, although also expressed as g m^{-3} or g kg^{-1} VS. Emissions were summed into weekly and whole trial total emissions for each tank. All averages are presented throughout with standard deviations (SD).

To compare the treatment effects between trials and treatments Cohen's d effect sizes were calculated on weekly total CH_4 (g m^{-2}) of treatments compared to controls:

$$\boldsymbol{Cohen's\ d = \frac{M_2 - M_1}{SD_{pooled}}} \qquad \text{Eq 7.1}$$

Where M_1 is mean CH_4 (g m^{-2}) of the control, M_2 is mean CH_4 (g m^{-2}) of the treatment, and SD_{pooled} is the pooled standard deviation.

The effect size (ES) is a standard way of showing the difference of the treatment from the control mean. The Cohen's d ES can thus be compared between studies and treatments. Average weekly total CH_4 was used for trials with treatment replicates for calculating the Cohen's d, while those that had no replicates were used as is. Some studies did not have standard 'controls' which is important to recognise. Trials H and I did not have manipulated treatments, but rather compared un-treated manures from different farms. In trial H the Cohen's d effect size was between manure with sand bedding and manure with sawdust bedding, while in trial I all tanks had manure from different farms with varying farm management. Additionally, in trial M the treatments were compared to fresh manure with inoculum instead of unmanipulated fresh manure.

Weekly total CH_4 (g m^{-2}), N_2O (g m^{-2}), and NH_3 (g m^{-2}) were cross-correlated with weekly air temperature (°C) for each tank and trial separately. Total CH_4 (g m^{-2}) from all tanks and trials together were correlated with TS (%), VS (%), pH, and inoculum (%). Lastly, total N_2O (g m^{-2}), and NH_3 (g m^{-2}) from all tanks and trials together were correlated with TS (%), VS (%), TAN (%), TN (%), pH, and inoculum (%).

Table 7.1 Studies performed at the Bioenvironmental Engineering Centre, Dalhousie University Agricultural Campus since 2006, year of study, associated manure farm source, monitoring length in days (d), and treatment studied.

Trial	Citation	Treatment	Year	Length (d)	Start Month	Farm	Volume (m^3)	Inoculum (%)	Fluxes	Missing data
A	VanderZaag et al. 2010	Winter Storage and agitation	2006	158	Dec	A	9.9	0	CH_4, N_2O, NH_3, CO_2	VS
B	VanderZaag et al. 2009	Straw Cover and agitation	2007	163	Jun	A	8.6	0	CH_4, N_2O, NH_3, CO_2	VS, manure temp*
C	VanderZaag et al. 2009	Biocap© cover	2008	162	May	A	8.6	0	CH_4, N_2O, NH_3, CO_2	
D	Wood et al. 2012	Dilution	2010	163	May	A	10.6	0	CH_4, N_2O, NH_3	mid-depth manure temp
E	Wood et al. 2014	Partial Emptying	2011	155	Jun	A	10.6	0	CH_4, N_2O, NH_3	mid-depth manure temp
F	Ngwabie et al. 2016	Inoculum	2012	171	Nov	A	10.6	0, 5, 10, 15, 20, 25	CH_4, N_2O, NH_3	
G	Ngwabie et al. 2016	Inoculum	2012	180	May	A	10.6	0, 5, 10, 15, 20, 25	CH_4, N_2O, NH_3	TAN
H	Le Riche et al. 2016	Comparing farms	2013	173	Jun	A - F	6.9	0	CH_4, N_2O, NH_3	
I	Le Riche et al. 2017	Wood vs sand bedding	2014	207	Apr	A, B	10.6	0	CH_4, N_2O, NH_3	manure temp
J	Le Riche et al. 2020	Inoculum	2015	274	Jan	B	10.6	0, 20	CH_4, N_2O, NH_3	air and manure temp
K	Sokolov et al. 2019	Inoculum and gradual filling	2016	130	Jun	B	11.4	0, 10, 20	CH_4, N_2O, NH_3	
L	Sokolov et al. 2019	Acidification	2017	162	Jun	B	10.6	0	CH_4, N_2O, NH_3	
M	Sokolov et al 2020	Acidification and inoculum	2018	145	Jun	B	10.6	20	CH_4, N_2O, NH_3	

* Manure temperature only available at 5 cm from the manure surface

7.3. Results and Discussion

7.3.1. Temperature

The average air temperature within the chambers was 17.3±1.32°C from all studies. The minimum average temperature was 15.1°C and maximum 20.1°C. The 30-year ambient air temperature normal as reported by Environment Canada (Debert, NS, ID 8201380) for months of April to October was approximately 5°C lower than inside the tanks (12.6°C). The clear plastic surrounding the chambers created a greenhouse effect, increasing the internal air temperature. Although a high rate of air exchange was set in the chambers (1-2 full air exchanges per minute), it was insufficient to keep the air temperature in the chambers equal to the ambient air temperature. Future research may improve air flow in the chambers by reducing static air pockets or further increasing air exchange rate in the chambers. In terms of this research, it is important to recognise that the GHGs from these tanks may be over-estimations compared to on-farm manure storages on the region.

Manure temperature at mid-depth (80 cm) was on average 17.0±0.89°C from all tanks and all studies. This was only slightly less than the average chamber air temperature. In most trials, the manure temperature between tanks was within ~1°C of each other. The only exception was Vanderzaag et al. 2010, which had one tank markedly higher than the rest (18.3°C vs 17.0°C) although temperatures were still within ~2°C, although not statistically different. This was likely an effect of the black, synthetic Biocap covers. There was no statistical difference in manure temperature due to treatment.

7.3.2. Manure Characteristics

The average fresh manure pH was 7.19±0.39 with the max of 8.20 and min of 6.70. Within trials the manure pH differed by 0.1-0.8 between tanks. Fresh manure with sawdust bedding had an average pH of 7.01±0.21 and manure with sand bedding had an average pH of 7.39±0.45

Table 7.2 Total methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3-N) emissions (g m^{-2}) and average chamber air and mid-depth manure temperature (°C) averaged (AVG), with standard deviations (SD), for all tanks over the entirety of each trial. Fresh manure pH, percent total solids (%TS), total ammoniacal nitrogen (%TAN), total nitrogen (%TN), and volatile solids (%VS) averaged over each tank.

	CH_4 g m^{-2}		N_2O g m^{-2}		NH_3 g m^{-2}		pH		Air Temp °C		Manure Temp °C		%TS		%TAN		%TN		%VS	
Trial	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
A	110	12.3	0.13	0.08	2.48	0.5	6.90	NA	0.92	NA	2.05	0.75	7.04	NA	0.12	NA	0.23	NA	NA	NA
B	2759	335	8.12	3.83	57.4	44.0	6.84	0.09	16.1	0.25	16.1	0.59	3.85	0.61	0.10	0.01	0.22	0.06	NA	NA
C	3878	74.2	8.12	4.45	33.9	25.5	7.02	0.04	16.5	0.14	15.7	0.46	3.05	1.51	0.09	0.02	0.17	0.04	2.44	1.33
D	8317	3461	12.8	10.5	168	87.8	6.90	0.06	15.2	0.17	15.8	0.35	4.72	3.73	0.12	0.04	0.31	0.18	3.90	3.08
E	1433	647	17.0	2.65	129	85.2	NA	NA	16.2	0.12	12.1	0.62	12.9	1.67	0.17	0.02	0.38	0.02	11.0	1.53
F	3789	930	20.3	7.86	450	129	8.00	0.30	17.6	NA	16.4	0.33	8.38	0.49	0.13	0.01	NA	NA	6.96	0.46
G	158	69.7	1.02	0.16	180	80.5	7.97	0.08	0.46	NA	3.8	NA	8.80	0.36	0.13	0.01	NA	NA	7.17	0.25
H	2647	1188	6.14	7.17	414	234	7.17	0.14	18.2	0.16	17.2	0.78	8.45	4.39	1.27	0.32	0.23	0.08	5.73	2.03
I	3968	1001	6.36	5.54	66.8	11.4	6.83	0.12	17.5	0.17	16.1	1.04	5.35	1.77	0.10	0.01	0.21	0.05	3.68	1.54
J	4008	1001	1.56	0.57	399	99.5	7.10	0.14	NA	NA	NA	NA	5.59	1.27	0.19	0.03	0.09	0.01	3.32	0.60
K	9701	1973	1.17	0.43	458	73.1	7.02	0.08	20.2	0.07	16.2	0.38	11.5	1.41	0.53	0.10	2.47	0.34	5.57	0.81
L	1506	1691	0.75	0.61	440	159	7.37	0.08	17.3	0.20	16.6	NA	21.9	0.61	0.19	0.02	0.46	0.02	9.24	0.74
M	2955	1607	2.65	164.0	392	77.2	7.70	0.17	18.2	0.12	15.3	0.25	12.4	0.53	0.07	0.02	0.27	0.02	6.04	0.15

($p<0.001$). Since these manures came from different farms, it is not possible to know if the difference was due to the bedding type or other farm factors such as feed, cow breed, etc.

The TS and VS varied greatly between studies. The average TS was 8.51±5.74%, with sand bedding manure having average TS of 11.3±5.91% and sawdust bedding manure average TS of 4.81±2.57%. Sand bedding had on average 58% ($p = 0.001$) more TS. However, Le Riche et al. 2016 (Figure 7.1 Study E) compared sawdust and sand bedding and reported statistically more TS ($p<0.001$) in sawdust bedding. Although all sand bedding manure came from the same farm, the %TS varied greatly going to >20% TS in 2017 (Sokolov et al. 2019). The difference in TS may have to do with timing of the last on-farm tank emptying where the manure was obtained or different farm practices. Year-round, on-farm sampling of TS from fresh manure may be needed to fully characterise the difference between the two bedding types. Additionally, sand bedding was described by Sokolov et al (2019) to have rapid sedimentation, which was not compared to sawdust bedding. LeRiche et al (2017) suggested that sawdust bedding may create more crust due to solids floating to the top rather than settling. Speed of sedimentation would change the effect of TS of GHG formation as it would change the %TS distribution throughout the manure volume.

The average VS was 5.21±2.42% (wet basis), with sawdust bedding manure having average VS of 5.09±2.23% and sand bedding manure average VS of 4.02±2.32%. The sand bedding had on average 21% more VS compared to the sawdust bedding ($p = 0.005$). On a dry basis, however, the sawdust bedding had higher VS by 28% ($p <0.001$), where sand bedding had 56.6% and sawdust bedding 78.2%. This shows that sawdust bedding increased the VS compared to TS, although sand bedding manure had overall less moisture content. This could be due to farm practices or other factors associated with sand bedding, such as higher evaporation.

The average TN was 0.49±0.72, with sawdust bedding manure having average TN of 0.23±0.10 and sand bedding manure average TN of 0.73±0.94. The sand bedding had on average 68% more TN, although not statistically different. The average TAN was 0.23±0.37%, with sawdust bedding manure having average TAN of 0.35±0.52% and sand bedding manure average TAN of 0.26±0.20%. The sawdust bedding had on average 34% more TAN, although not statistically different.

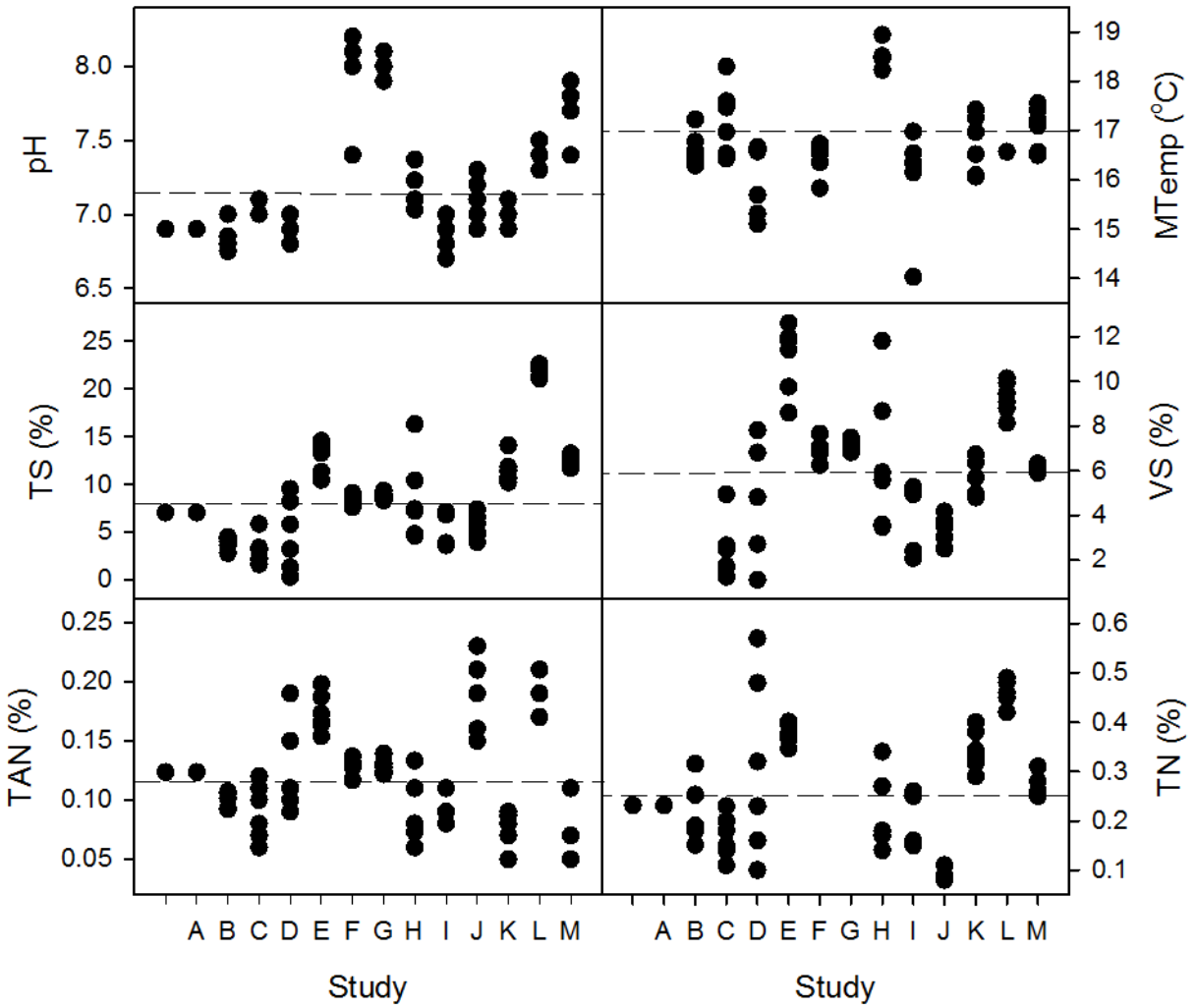

Figure 7.1 Starting manure characteristics, pH, total solids (TS, %), volatile solids (VS, %), total ammoniacal nitrogen (TAN, %), total nitrogen (TN, %), and average manure temperature at mid-depth (MTemp; °C) over the entire monitoring period from each trial. Horizontal line indicates parameter average over all studies.

7.3.3. Treatment Results

Most manure practices tested had <50% CH_4 reductions, these include covers, reducing TS/VS, and removing inoculum. In fact, covers and inoculum removal (compared to 5 – 25% inoculum) all had <32% CH_4 reductions. The best CH_4 emission reductions were from using H_2SO_4 to acidify manure, with >77% reductions. Figure 2 shows a radar graph of the Cohen's d effect size for each treatment. The ES is a standard way of showing the difference of the treatment from the control mean. The Cohen's d ES can thus be compared between studies and treatments. Most treatments had an ES ≤ 2. The largest ES of 6.03 and 5.84 were from 2.4 and 1.4 L H_2SO_4 m^{-3} manure treatments, respectively, from Sokolov et al (2017). The follow-up study which had 1.2 L H_2SO_4 m^{-3} manure with inoculum had an ES of 3.09, which follows the lower CH_4 emissions reported (Sokolov et al. 2020b).

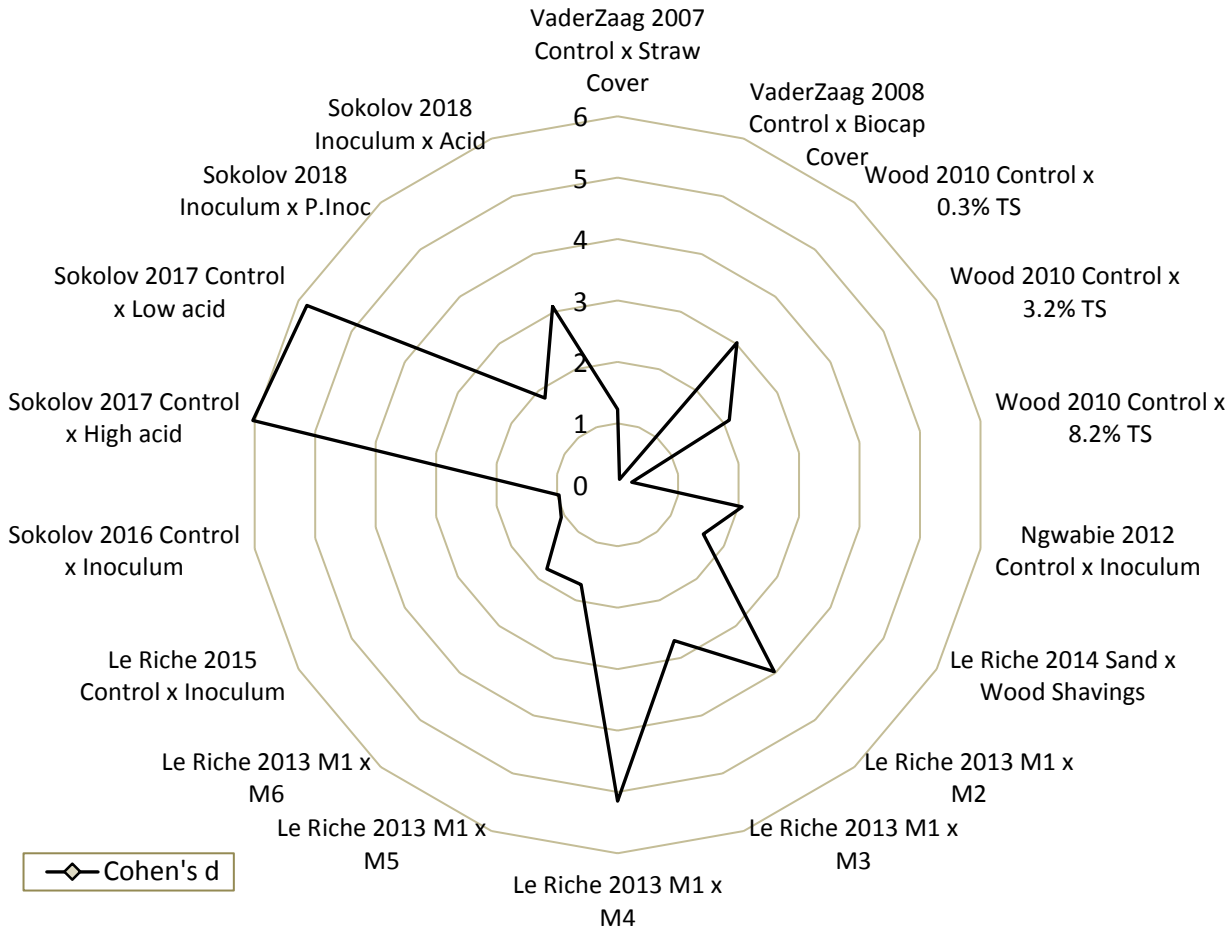

Figure 7.2 Difference between treatment means expressed as Cohen's d effect size of total weekly methane (CH_4) emissions (g m^{-2}) comparison of treatments and controls from trials B, C, D, G, K. Comparison of manure from farm M1 containing sand bedding with manures from farms M2, M3, M4, M5, and M6 with sawdust bedding from trial H. Comparison of sand and sawdust bedding manures from trial I. Lastly, comparison of previously acidified inoculum (P.Inoc) and inoculated control (Inoculum) from trial M.

Surprising, there were high ESs of 5.15, 3.97, and 2.70 comparing untreated manures from different farms, reported by Le Riche et al. (2016). These farms used different bedding, barn cleaning techniques and had different resulting manure characteristics. Farm D (ES = 5.15) had surprisingly low CH_4 emissions, although it is unclear why this was. This highlights how important in-barn practices can be on influencing GHG emissions from stored manure. Future research should focus on characterising these practices to develop best management practices.

Reducing N_2O and NH_3 is less critical as they only contribute <10% of the total GHG emissions on a CO_2 basis. However, many practises were able to reduce NH_3 by up to 89%, while N_2O was increased in certain cases by up to 60%.

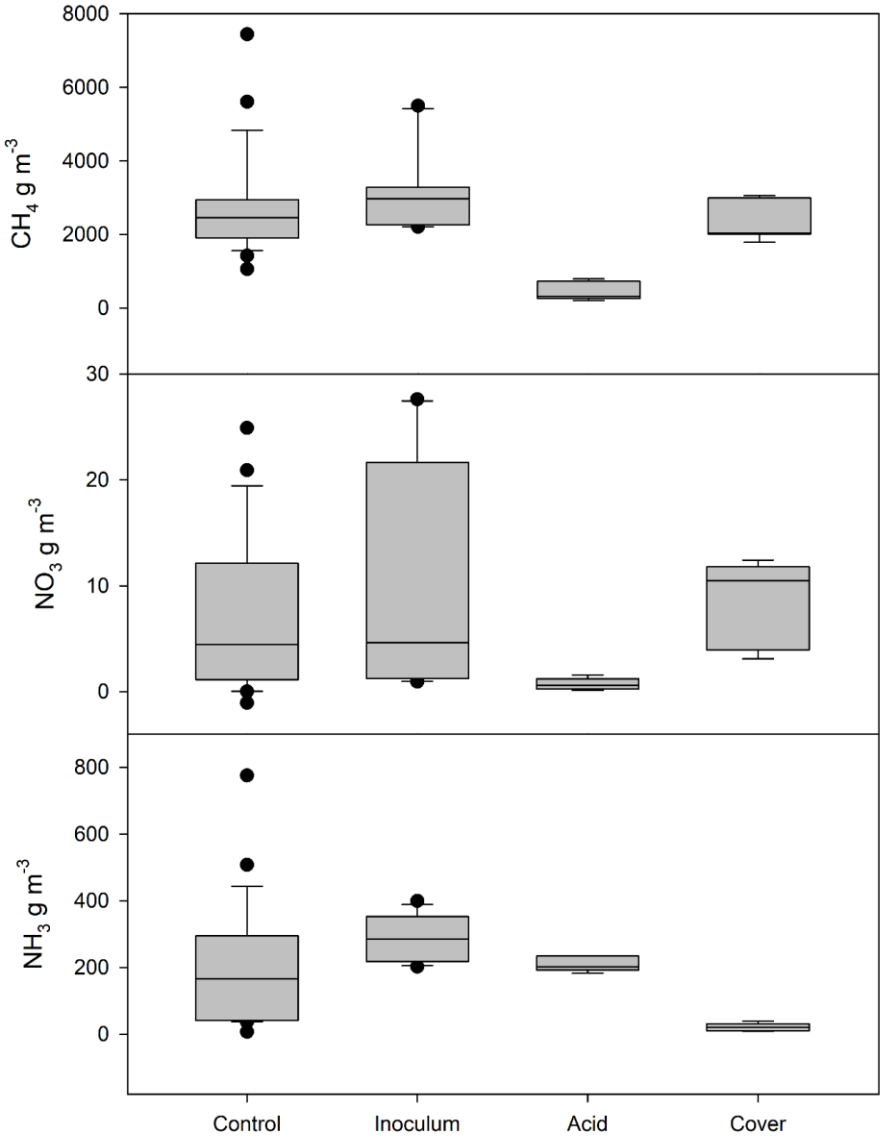

Figure 7.3 Box plot of CH_4 (g m^{-3}), N_2O (g m^{-3}), and NH_3 (g m^{-3}) for all tanks over all years with inoculum (5 – 25%), acid, or cover treatment and controls (controls contain only FM without inoculum, acid, or covers) from studies A – J.

Covering manure to physically block GHG emissions was the first treatment tested at the site (VanderZaag et al. 2009, 2010b). Both natural, straw and synthetic, Biocap permeable synthetic covers were able to reduce emissions. Straw covers reduced CH_4 emissions by 19% and NH_3 by

69%, although increased N_2O by 60%. Synthetic covers reduced N_2O emissions by 68%, NH_3 by 89%, although had no effect on CH_4. Given that CH_4 was unaffected by Biocap covers, the total emissions were not markedly affected.

To test the effect of VS and TS on biogas emissions, Wood et al. (2009) studied dilution, Le Riche et al. (2017) tested sand versus sawdust bedding in manure, and Le Riche et al. (2016) compared manures from 6 different farms. The dilution showed increasing CH_4, N_2O, and NH_3 emissions as a factor of increasing TS. With 50% less TS, there was 50% less CH_4 and N_2O, and 60% less NH_3. However, due to the increased volume of manure as a result of dilution, it is not certain if these reductions would scale up. Larger storages would be required, and more manure volume would create more GHGs, depending on the storage depth, surface area to volume ratio, and storage retention time. In fact, CH_4 production on a per VS basis increased correspondingly with increasing dilution (decrease in %TS and VS). Undiluted manure produced 94% fewer CH_4 emissions compared to the largest dilution (115 and 1805 g kg^{-1} VS, Table 7.4). Le Riche et al. (2017) reported that sawdust bedding manure had higher TS (6.6% v 3.9%) and VS (4.8% v 2.6%), and produced 51% more CH_4, 88% more N_2O, and 13% more NH_3. On the other hand, Le Riche et al. (2016) compared manures from 6 farms, one of which had sand bedding. The sand bedding manure had appreciatively more TS (16.3±0.21% vs average 6.88±2.22%) although comparable VS (7.19±0.28% vs average 5.44±1.98%). However, manure with sand bedding produced on average 2× the CH_4 as the sawdust bedding manures. Sawdust bedding contributes both TS and VS (average 78.0%) to the manure. In the case of sand bedding, it contributes little organic matter (44.2% VS of TS), therefore the TS contributes little carbon or nutrients to the manure. Straw or wood bedding, on the other hand, contributes both carbon and nutrients, increasing the TS and VS.

Four studies compared stored manure with inoculum versus manure with no inoculum. Wood et al. (2014) compared GHGs from stored manure with no inoculum and stored manure with 50% inoculum. This was intentionally an extreme amount of inoculum to characterise a worst-case effect of inoculum. They reported 56% reduction in CH_4 in tanks with no inoculum compared to inoculated tanks. Ngwabie et al. (2016) reported that CH_4 emissions increased correspondingly with inoculum amount, resulting in a 25% reduction in CH_4 when inoculum levels were reduced from 15% to 5%. Le Riche et al (2020) reported 34% less CH_4 from manure with no inoculum compared to manure with 20% inoculum. Lastly, Sokolov et al. (2019) reported average 24% less CH_4 from manure with no inoculum compared to manure with 10% and 20% inoculum. Sokolov et al. (2019) also reported that gradual filling, compared to batch filling, manure tanks with inoculum further increased the CH_4 by 27%. All three studies reported no clear effect of inoculum on N_2O and NH_3.

Acidification had the best treatment results of all manure practices tested. Sokolov et al (2019) reported application of H_2SO_4 after storage filling reduced overall CH_4 emissions by 88% and NH_3 by 47%. A follow-up study with 20% inoculum reported a 77% reduction of CH_4 and 33% reduction NH_3 with H_2SO_4 application. Additionally, using manure acidified 1 y prior as inoculum reduced CH_4 by 38% and NH_3 by 23% compared to fresh manure with untreated inoculum.

7.3.4. Methane

7.3.4.1. Production Lag and Peak

Methane production in most cases followed a similar pattern with 3 distinct phases throughout the season: lag phase, growth phase, and die-off phase (Figure 7.4, Figure 7.5, Figure 7.6). The lag phase was on average 50±15 d in length with the start of the trial being between April and June. The growth phase was on average 35±27 d in length, with the highest emissions usually

around July and August. The die-off phase would continue until the end of the monitoring period. The lag phase length and growth phase length were correlated, suggesting that the rate of microbial growth in the lag phase influenced the rate of microbial growth in the growth phase. There was no clear correlation of lag phase length or growth phase length with total CH_4 emissions. This is surprising, as total CH_4 production should be correlated with the production rate. However, this may suggest that the microbial density of at the start of storage (i.e. inoculum) may be a greater predictor of total CH_4 production, rather than the growth rate throughout storage.

1.1.1.1. Temperature

It is known that methane production increases with temperature, however, correlation of total CH_4 (log_{10} g m^{-2}) with average manure temperature (°C) from all trials was poor ($r = 0.248$). Correlation with average air temperature (°C) from all trials was slightly worse ($r = 0.082$). Manure temperature has a depth gradient within the storage tanks, with variable temperature near the manure surface and more consistent temperature near the storage bottom. Although there was only one depth of manure temperature measurement, it was still a better predictor of total CH_4 production than air temperature. More consistent manure temperature measurements at multiple depths may provide a better correlation and prediction of total CH_4.

Table 7.3 Whole trial average methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3) emissions (g m^{-2}) from each treatment, sawdust bedding control and sand bedding control. Showing the average trial length in days (d) and N denotes the number of tanks that were used to calculate the average.

		CH_4 g m^{-2}		**N_2O g m^{-2}**		**NH_3 g m^{-2}**			**Storage d**	**Studies**
		Avg	**SD**	**Avg**	**SD**	**Avg**	**SD**	**N**	**Avg**	
Control	Wood shavings	3360	2545	11.43	7.13	191	146	18	171.6	A- I
	Sand bedding	4053	2054	1.33	0.96	376	262	10	207	H - M
	All	3707	2300	6.38	4.04	284	204	18	184.2	A - M
	Gradual fill	6470		1.16		467		1	130	K
Acidification	1.2 + inoculum	1196	106	1.2	0.54	325	3.37	2	145	M
	1.4	491	71.8	0.34	0.05	376	2.03	2	162	L
	2.4	388	89	0.6	0.67	304	14.9	2	162	L
Previously acidified inoculum		3258	914	2.08	0.57	370	60	2	145	M
Inoculum	5	3539		27.1		341		1	163	G
	10	6452	3712	14.3	18.9	608	46.7	2	146.5	G, K
	10 + gradual fill	12095		1.95		473		1	130	K
	15	4768		7.99		577		1	163	G
	20	5366	1918	5.61	8.36	442	84.3	7	200.7	G, J, K, M
	20 + gradual fill	11394		0.92		473		1	130	K
	25	3555		13.9		348		1	163	G
	50	1980	341	16.7	2.21	56.7	8.4	3	155	E
Straw cover	15	2484	229	8.81	3.1	39.3	17.6	2	163	B
	30	2633	28.4	11.5	1.38	26.2	20.3	2	163	B
Biocap cover		3895	89.8	6.29	4.79	23.3	15.4	3	162	C
Dilution	8.2% TS	12581		25		262		1	163	D
	5.8% TS	8592		15.5		193		1	163	D
	3.2% TS	7233		5.06		149		1	163	D
	1.3% TS	3746		2.34		70.8		1	163	D
	0.03% TS	5798		3.82		69.2		1	163	D

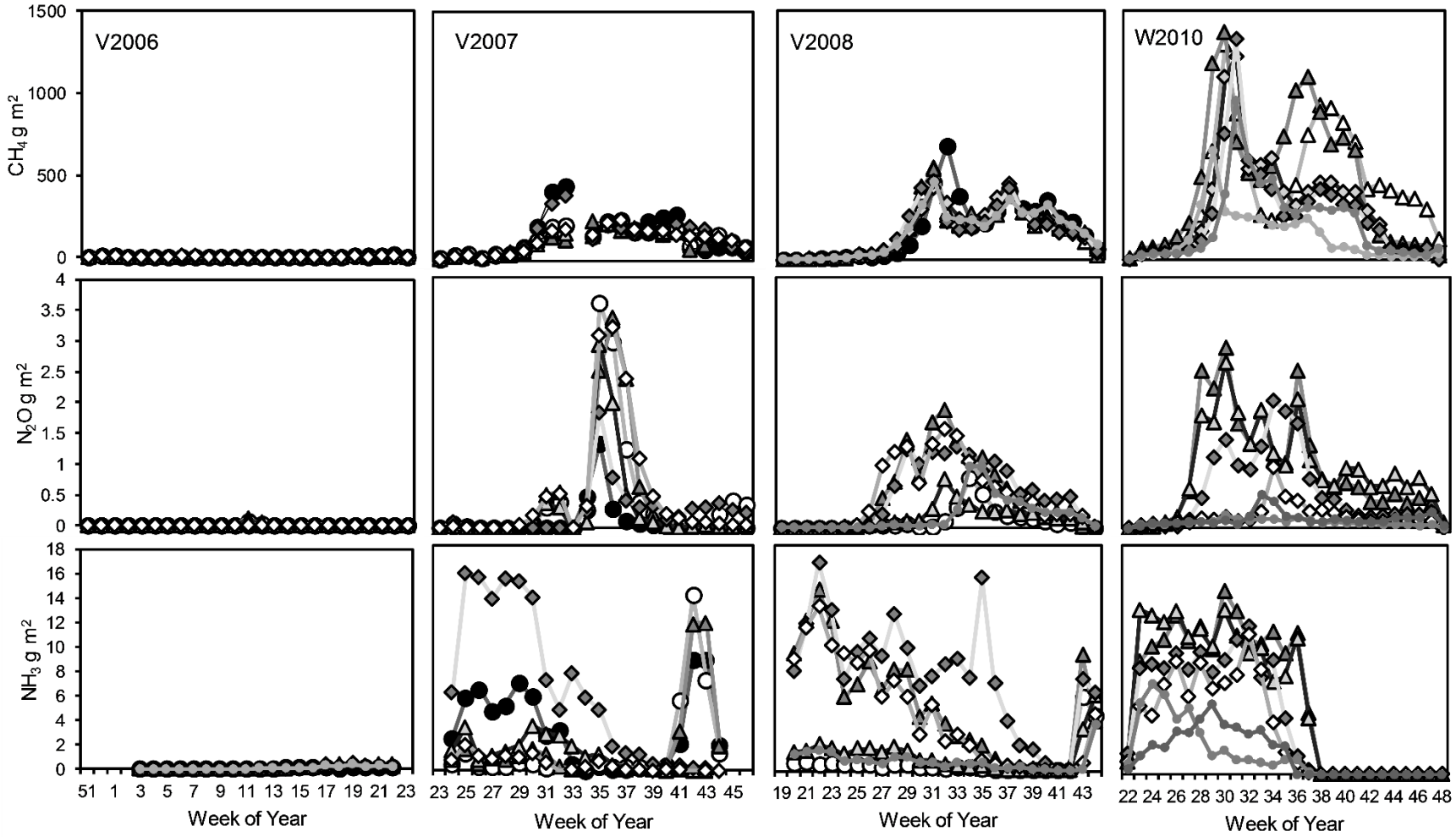

Figure 7.4 Weekly total methane (CH_4 g m^{-2}), nitrous oxide (N_2O g m^{-2}), and ammonia (NH_3-N g m^{-2}) from all tanks of trials A – VanderZaag et al. 2010 (V2006), B – VanderZaag et al. 2009 (V2007), C – VanderZaag et al. 2009 (V2008), and D – Wood et al. 2012 (W2010).

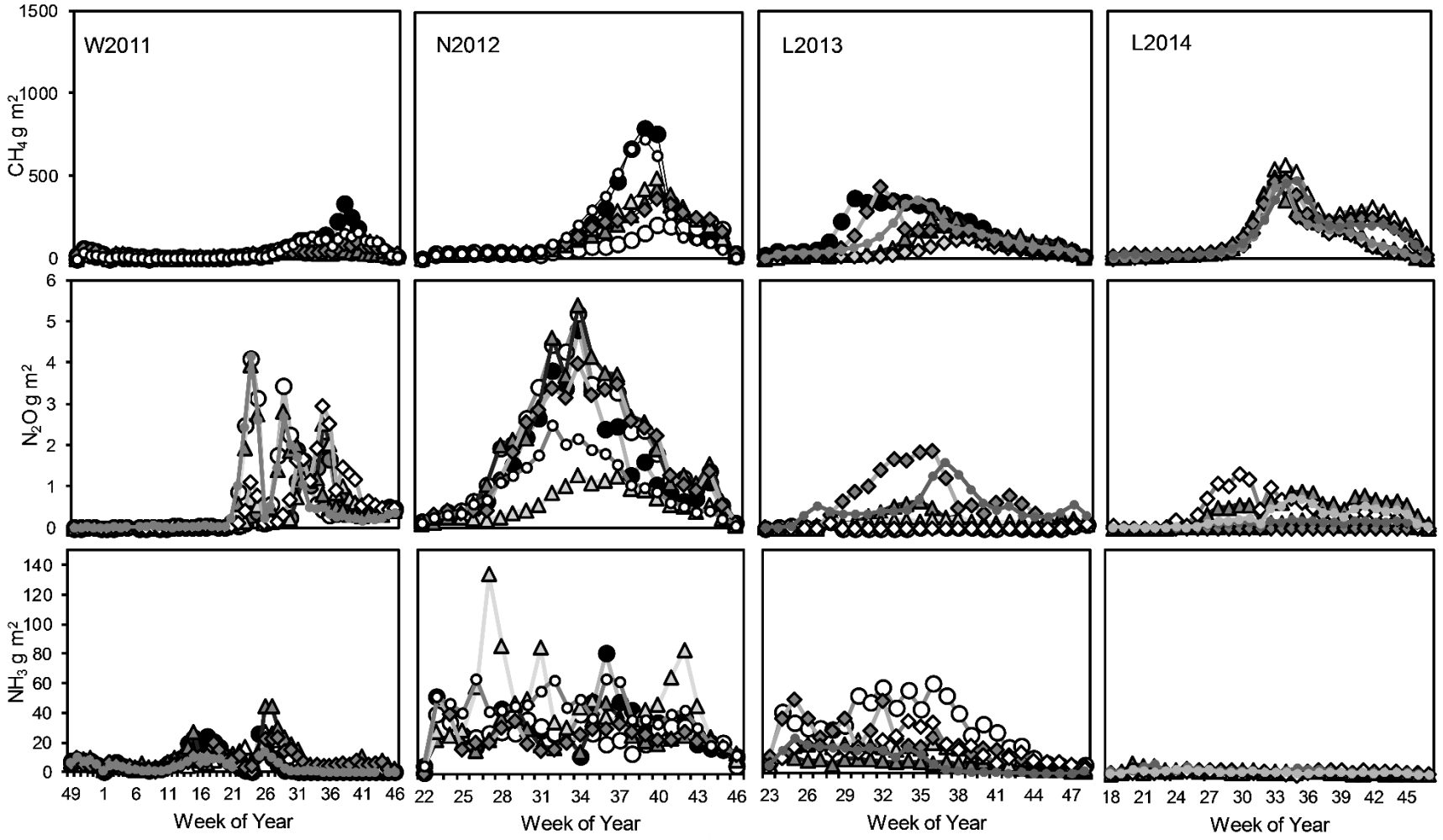

Figure 7.5 Weekly total methane (CH_4 g m^{-2}), nitrous oxide (N_2O g m^{-2}), and ammonia (NH_3-N g m^{-2}) from all tanks of trials E – Wood et al. 2014 (W2011), G – Ngwabie et al. 2016 (N2012), H – LeRiche et al. 2016 (L2013), and I – LeRiche et al. 2017 (L2014).

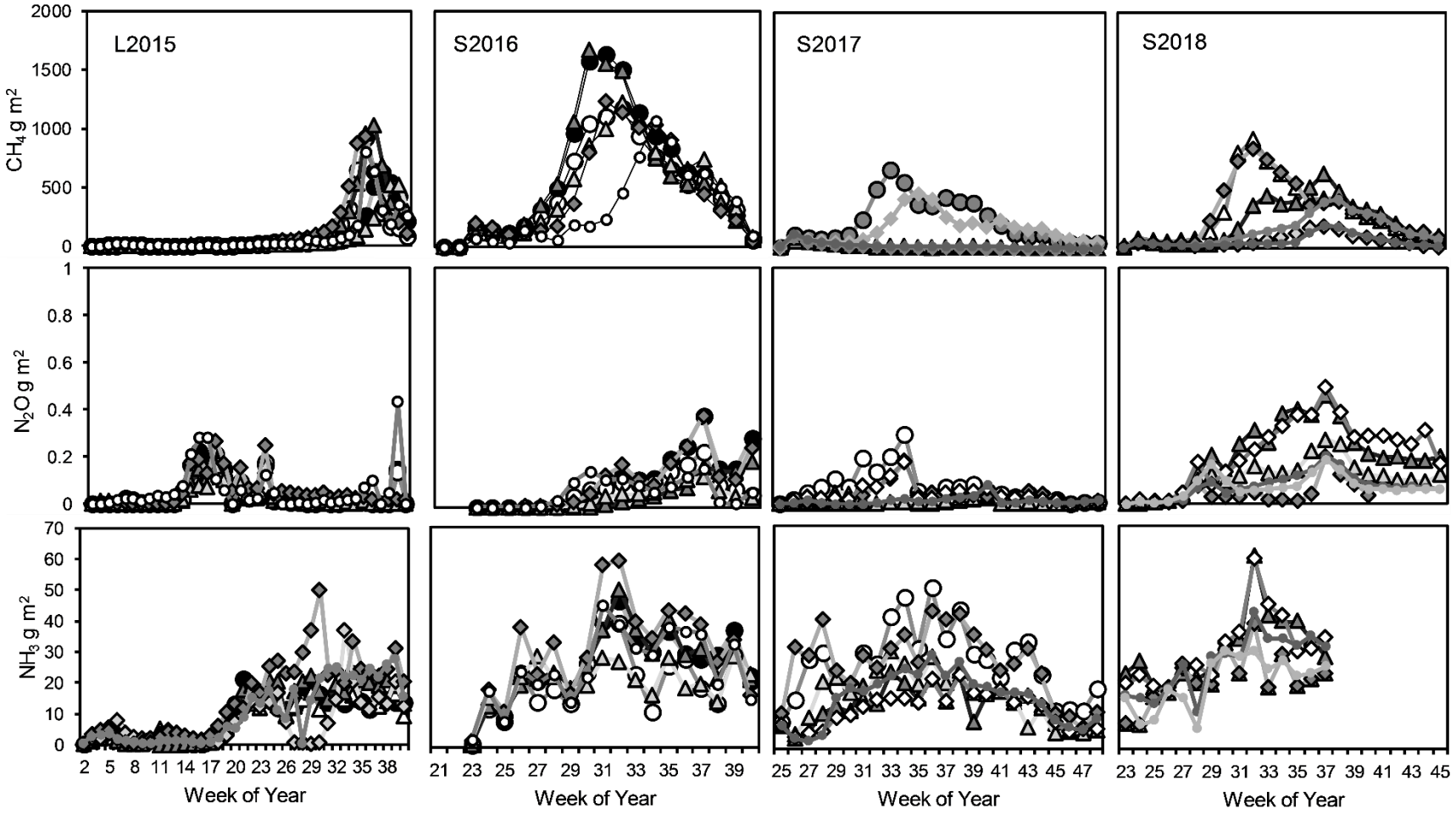

Figure 7.6 Weekly total methane (CH_4 g m^{-2}), nitrous oxide (N_2O g m^{-2}), and ammonia (NH_3-N g m^{-2}) from all tanks of trials J – LeRiche et al. 2020 (L2015), K – Sokolov et al. 2019 (S2016), L – Sokolov et al. 2019 (S2017), M – Sokolov et al. 2020 (S2018).

Air temperature was used for weekly correlations, due to more consistent measurements cross trials and a more complete data set. Weekly total CH_4 (log10) also had poor correlation with weekly average air temperature ($r = 0.222$), therefore cross-correlations were performed on each storage tank from each trial separately. An offset on average of 4.6±2.4 weeks increased the correlation to $r = 0.36 – 0.944$. It was unclear why some storage tanks had a higher off-set than others, although it may relate to the length of the lag. Le Riche et al. (2016) compared pre- and post-peak linear regressions of daily average CH_4 ($g\ m^{-2}$) and temperature. They reported a moderate correlation post-peak (i.e. during the die-off phase; $R^2 = 0.58$) and no meaningful correlation pre-peak (i.e. lag and growth phases; $R^2 = 0.01$).

It was expected that the acidified treatments from Sokolov et al. (2017) and Sokolov et al. (2018) might have different air temperature responses, as the CH_4 production did not exhibit the typical CH_4 production curve, however, in both studies all tanks had $r > 0.82$. The lowest correlations were from Vanderzaag et al. (2009) and Sokolov et al. (2019). Vanderzaag et al. (2009) studied straw covers and agitation and had correlations of $r = 0.36 – 0.71$, while Sokolov et al. (2019) studied gradual versus batch filling with or without inoculum and had cross-correlations of $r = 0.50 – 0.61$. This suggests that the manure temperature in covered tanks is less representative of the surrounding air temperature, which may influence CH_4 production response. Additionally, in the case of Sokolov et al. (2019), at each tank filling the temperature gradient within the tanks may have been disrupted as new manure was added to the tank.

1.1.1.2. Total and Volatile Solids

Manure samples of fresh manure were taken consistently from the start of each trial and only in some studies throughout the trial. Sampling throughout the trial was less accurate, as with temperature, a depth gradient of TS and VS was created in the storages due to sedimentation

which occurred rapidly. Therefore, total CH_4 production was correlated with initial TS and VS, rather than correlations over time. Correlation of total CH_4 with TS (r = -0.434) and VS (r = -0.444) from all tanks and trials was moderate to low. Doing separate correlations of tanks containing manure with sawdust bedding and tanks containing manure with sand bedding had marginally better correlations. Sawdust bedding and total CH_4 had negative correlations with TS (r = -0.412) and VS (r = -0.473). Sand bedding and total CH_4 also had negative correlations with TS (r = -0.512) and VS (r = -0.444). Individual study correlations of CH_4 with TS and VS were highly variable with r = 0.20 – 0.94 for TS and r = 0.03 – 0.94 for VS. The best correlations were from Wood et al. (2012) (r = 0.94 for both TS and VS) and LeRiche et al. (2017) (r = 0.91 for both TS and VS). Wood et al. (2012) studied dilutions (TS = 0.3 – 9.5% and VS = 0.2 – 7.8%), while LeRiche et al. (2017) compared sand versus wood shaving bedding (TS = 3.60 – 7.13% and VS = 2.07 – 5.26%). This suggests that TS and VS may affect CH_4 production, however only on a larger scale. Studies which used the same manure had small difference in TS and VS between tanks which did not affect the CH_4 in a meaningful manner.

1.1.1.3. pH

The total CH_4 (log g m^{-2}) correlated poorly with pH, when looked at all treatments (r = 0.204) as well as controls only (r = -0.234). The acidification studies, Sokolov et al. (2019) and Sokolov et al. (2020) also had a poor correlation (r = 0.22), suggesting that pH is a poor predictor of the treatment effect of using H_2SO_4. It has been suggested that H_2SO_4 reduces CH_4 production through sulphur transformations rather than lower pH (Petersen et al. 2012). The pH values achieved in these studies were not substantially lower than FM pH (6.0 – 7.0). As with TS and VS, perhaps larger differences are necessary to see CH_4 effects due to pH changes. Future research should test different acids and lower pH ranges.

1.1.1.4. Inoculum

Methane produced from all control tanks through all trials was on average 3.71±2.30 kg m^{-2}, which was 13% less than the average CH_4 produced from tanks containing inoculum (4.28±1.58 kg m^{-2}). Inoculum amount (%) and total CH_4 (log g m^{-2}) had poor correlation (r = -0.019) although this is likely due to not enough range in inoculum amount and not enough data points for a good correlation. More data points are especially important for manure research due to high manure variability. Ngwabie et al. (2016) monitored tanks with 0, 5, 10, 15, 20, and 25% inoculum. With the 25% inoculum data point removed, the total CH_4 (log g m^{-2}) had a good correlation with inoculum amount (r = 0.925). The 25% inoculum total CH_4 was much lower than expected, which may be due to reduced effect of inoculum with increasing amount, or due to measurement error.

1.1.2. Nitrous Oxide and Ammonia

There was no consistent N_2O emission trend over time. The timing of peak N_2O emissions over all tanks and studies ranged widely from week 5 to 25 of study (April – November). Weekly total N_2O emissions correlated poorly with weekly average air temperature. Although some tanks had great correlation (up to r = 0.89), most had a moderate correlation (approx. r = 0.5-0.6) and some low or negative correlations (r < 0.2).

Total N_2O (log10 g m^{-2}) emissions had poor correlation with initial %TS (r = 0.313), %VS (r = 0.103), %TN (r = 0.002), and %TAN (r = 0.028). There also a poor correlation with initial pH (r = 0.059).

Ammonia emissions tended to peak within the first 10 weeks of the study, often prior to peak air temperature. This was likely due to high TAN at the start of the storage and no crust had yet formed. Following the peak, temperatures usually slowly declined till the end of the monitoring

period. Weekly total NH_3 emissions correlated moderately with weekly average air temperature (average r = 0.39).

Total NH_3 emissions had moderate correlation with initial %TS (r = 0.530), %VS (r = 0.343), but poor correlation with initial %TN (r = 0.298), and %TAN (r = 0.234). Initial pH of manure correlated moderately with total NH_3 (g m^{-2}; r = 0.528) which aligns with Sokolov et al (2019) who reported 47% NH_3 reduction from acidification of manure.

Inoculum presence appeared to influence NH_3 emissions. Control tanks produced on average 44% less NH_3 emissions compared to tanks with inoculum (267±194 and 475±97.6 g m^{-2}; Table 7.4). However, individual trials did not observe a clear influence of inoculum on NH_3 emissions. Only LeRiche et al (2020) clearly observed high NH_3 emissions with inoculum presence, although difference was not notable.

1.2. Recommendations and Future Work

The warm-season trials in 2006 – 2018 produced on the average 3,975±2,7585 g CH_4 m^{-2} $trial^{-1}$ (94.8±231 g CH_4 Kg^{-1} VS $trial^{-1}$), 7.83±7.94 g N_2O m^{-2} $trial^{-1}$ (1.86±1.89 g N_2O Kg^{-1} TN $trial^{-1}$ and 4.60±4.38 g N_2O Kg^{-1} TAN $trial^{-1}$) and 270±200 g NH_3 m^{-2} $trial^{-1}$ (79.9±88.7 g NH_3 Kg^{-1} TN $trial^{-1}$ and 173±163 g NH_3 Kg^{-1} TAN $trial^{-1}$). There were 3 trials which stored manure over winter (trial A and F, and unreported data from manure later used as inoculum for trial E). These trial produced on average 226±156 g CH_4 m^{-2} $trial^{-1}$ (17.2±19.0 g CH_4 Kg^{-1} VS $trial^{-1}$), 2.60±3.94 g N_2O m^{-2} $trial^{-1}$ (0.72±0.49 g N_2O Kg^{-1} TN $trial^{-1}$ and 2.12±2.01 g N_2O Kg^{-1} TAN $trial^{-1}$) and 119±101 g NH_3 m^{-2} $trial^{-1}$ (57.4±39.2 g NH_3 Kg^{-1} TN $trial^{-1}$ and 196±188 g NH_3 Kg^{-1} TAN $trial^{-1}$). Based on these averages, over the 12 yr of research, CH_4 emissions over winter contributed on average <5% of the total yearly CH_4 emissions. Winter storage produced fewer average CH_4 emissions than any treatment. Although it is not possible to cool manure throughout the year,

maximizing winter storage over summer storage whenever possible will help reduce overall emissions.

Table 7.4 Average of all tanks from each treatment of methane (CH_4 g) emissions as a basis of initial fresh manure volatile solids (VS Kg), nitrous oxide (N_2O g) emissions on a basis of initial fresh manure total nitrogen (TN Kg) and total ammoniacal nitrogen (TAN Kg), and ammonia-N (NH_3-N g) on a basis of initial fresh manure TN and TAN.

		CH_4 g kg^{-1} VS		**N_2O g kg^{-1} TN**		**N_2O g kg^{-1} TAN**		**NH_3 g kg^{-1} TN**		**NH_3 g kg^{-1} TAN**		
		Avg	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**N**
Control	Wood shavings	59.3	53.5	3.07	1.92	6.88	4.13	63.4	66.5	142	141	16
	Sand bedding	64.6	24.0	0.58	0.54	0.66	0.64	130	103	178	159	10
	All	61.3	43.9	2.14	1.97	4.66	4.48	88.2	86.5	155	146	26
	Gradual fill	78.2		0.17		0.77		67.6		312		1
Acidification	1.2 + inoculum	12.4	1.18	0.26	0.13	0.94	0.67	68.8	4.23	236	71.9	2
	1.4	3.2	0.35	0.04	0.01	0.11	0.02	49.4	1.94	123	0.66	2
	2.4	2.6	0.85	0.08	0.09	0.20	0.22	42.0	2.06	106	13.5	2
Previously acidified inoculum		33.1	7.72	0.50	0.14	2.30	1.13	88.6	14.4	387	29.0	2
Inoculum	5	31.2				12.8				161		1
	10	63.2	41.6	0.19		6.82	8.13	115		479	264	2
	10 + gradual fill	142		0.33		1.41		79.8		342		1
	15	42.6				4.25				307		1
	20	69.2	23.3	0.96	0.48	3.66	4.36	210	143	297	213	7
	20 + gradual fill	133		0.17		0.76		86.5		391		1
	25	35.5				7.39				186		1
	50	10.2	1.98	2.75	0.32	6.48	1.03	9.3	1.37	22.0	4.04	3
Straw cover	15			3.80		6.92		17.2		31.0		2
	30			3.74		8.88		10.1		21.2		2
Biocap cover		158	68.9	3.70	3.93	6.76	7.22	13.5	13.1	24.6	24.2	3
Dilution	8.2% TS	115		3.25		10.4		34.0		109		1
	5.8% TS	111		3.02		10.7		37.6		134		1
	3.2% TS	167		1.37		3.15		40.3		92.7		1
	1.3% TS	212		0.91		1.33		27.6		40.1		1
	0.03% TS	1805		2.38		2.65		43.1		47.9		1

The main mitigation strategies tested were covers, removal of inoculum, and acidification. Only cover method to reduce CH_4 emissions was using straw, with reductions up to 30%. Although these reductions were relatively low, covering manure with straw is a simple treatment method, which is easy and inexpensive for farmers to implement on-farm. However, it is important to note that on-farm manure storages predominantly contain inoculum. Therefore, the inoculated manure emissions are more comparable to actual on-farm emissions than the untreated control

emissions. Since covers were tested on manure without inoculum, the results may be over- or under-representative of actual reductions seen on-farms. Future research may consider testing straw cover on manure with inoculum presence for more representative treatment effects.

Presence of inoculum increased CH_4 emission by up to 2 × compared to untreated manure, depending on the trial and amount of inoculum. This would be ~50% reduction in CH_4 emissions due to complete storage cleaning. However, these reductions were in comparison to the higher amounts of inoculum (20, 25, and 50% inoculum). Many farms may have lower levels of inoculum, which would reduce the effectiveness of further cleaning. Although unlike covering and acidification which require additional inputs into the storages, removing manure is already a process being done by farmers. The better this existing process can be performed, the lower the emissions will be. This method of GHG reduction may be harder for farmers to clearly quantify if they want to claim carbon credits for GHG reduction, as it would require quantifying the extra manure removed. Additionally, inoculums may have different inoculating effects depending on the manure type, age, and when the emptying occurs. These effects have yet to be properly characterized, therefore the best way to quantify the cleaning effects, is to measure the methanogen abundance and activity, which is not feasible for farmers.

Acidification was tested in trial L comparing acidified manure without inoculum to untreated control manure and in trial M comparing acidified manure with inoculum compared to inoculated manure. In both cases, acidification had the best treatment results to date from all BEEC trials. Reduction of 73-89% CH_4 were markedly better than other treatments tested. H_2SO_4 performed exceedingly well, although the fate of sulphur applied to the manure has not yet been examined. The usefulness of H_2SO_4 for mitigating GHGs is promising and could lead to

an easy carbon credit calculation. More research is required to be certain there are no health threats to humans and animals with possible sulphur emissions.

Other manure management practices have been explored to quantify effect on GHG emissions, mostly notable being comparison of sawdust and sand bedding addition to manure. Comparing sawdust to sand bedding had mixed results between trials, but overall average CH_4 emissions were 17% lower from sawdust. It was expected that the higher %VS from sawdust would increase CH_4 production, therefore another mechanism must be increasing the CH_4 production in sand bedding manures. However, overall emission differences are very small, therefore not enough to recommendation changing farm practices. Future research should focus on exploring other farm management practices which may influence GHG emissions, such as cow breed, diet, barn cleaning, etc.

Changes in methods and experimental designs over the last 12 years limit the ability to make broad comparisons. Changes in manure type, placement of manure thermocouples, and number of treatment replicates reduces the accuracy of between trial comparisons. Future work should focus on standardizing the methods at BEEC with more focus on continuity between years.

1.3. Main Results

- Untreated, control manure had large variability in emissions, producing on average 3707±2300 g CH_4 m^{-2} $trial^{-1}$, 6.38±4.04 g N_2O m^{-2} $trial^{-1}$, and 284±204 g NH_3 m^{-2} $trial^{-1}$.
- Acidification with H_2SO_4 had the best treatment effects, with reductions of 73 – 89% CH_4, 58-76% N_2O, and 33-53% NH_3. More research is needed to determine potential hazards of sulphur emissions from H_2SO_4 treatment.
- Removal of inoculum on average reduced emissions of CH_4 (g m^{-2} $trial^{-1}$) by 38% and NH_3 (g m^{-2} $trial^{-1}$) by 44%.

- Straw covering reduced average emissions by 31% CH_4 (g m^{-2} $trial^{-1}$), and NH_3 (g m^{-2} $trial^{-1}$) by 89%. This strategy is simple and inexpensive for farmers to implement and may be a good strategy when others are not available. More research is needed to see interactions with inoculum and gradual filing.
- Poor correlations of TS or VS with CH_4 productions suggests that only large differences in either can markedly affect CH_4 production. More research into other manure management practices, such as cow diet, breed, barn cleaning, etc. is needed to develop best manure management practices.

2. Conclusion

2.1. Research Overview

Mitigating GHG emission from dairy farming is increasingly important as Canadian population is projected to increase to 51 million people in the next 50 years with a corresponding demand on agriculture production (Statistics Canada 2014). Manure storages source only a fraction of dairy GHG emissions, although have the benefit of being a contained emission source. This means that GHG production from manure storages can be mitigated with the right practices. This thesis contributed to manure storage GHG mitigation knowledge with a focus on effective practices which are inexpensive and requiring few resources.

More concretely, this thesis has contributed to the advancement of reducing GHG emissions from liquid dairy manure through original research by: i) testing gradual and batch fillings methods with inoculum stored manure ii) field-scale and lab-scale studies of dairy manure acidification, and iii) a quantitative and qualitative review of 12 years of research from a meso-scale manure storage facility.

2.2. Contribution to Knowledge

Most of this thesis addresses different acidification practices at the lab or meso-scale. Acidification had yet to be tested in a Canadian setting, which is important as different management systems may pose unique challenges and GHG emissions. This research focused on using smaller amounts of acid and less frequent acidification to reduce the cost of the practice, while still reducing emissions. This was a novel approach, as previous acidification research focused on low resulting pH and infrastructure for thorough mixing of acid and manure. A simple alternative to large manure mixing infrastructure was designed with the intent of reducing the cost of on-farm acidification. This included applying acid with a peristaltic pump with acid-

resistant tubing in the meso-scale trials. Acidification was also studied in relation to inoculum, to reduce frequency of acidification or an alternative to tank cleaning. This was novel, as previous research has focused on acidifying all fresh manure, rather than only a portion. Acidifying only the inoculum gave similar emissions to having no inoculum, suggesting that this may be an alternative to complete tank cleaning. Additionally, aged acidified manure exhibited a reduced inoculating ability, suggesting a long-term treatment effect of acidification. The long-term GHG reduction effect of acidification had not yet been tested and this research suggests that it may allow for less frequent acidification.

Inoculum removal has been previously studied and shown to reduce CH_4 production. The novelty of this research was the addition of gradual tank filling to explore result bias in batch-filling meso-scale methods and its interaction with inoculum amount. This study showed that containing inoculum produced more total GHG emissions when filled gradually. Therefore, previous inoculum research using batch filling may be underrepresenting the treatment differences.

Lastly, this thesis contains a quantitative and qualitative review of 12 years of research from an experimental, meso-scale manure storage site. A novel aspect of this study was the large number of 6-month trials from the same research site. Unlike a meta-analysis, this dataset has more ability for direct comparison. It uniquely offers researchers an easily accessible synthesis of 12 years of manure management research to inform future research and best manure management practices. Overall, acidification showed the best treatment ability, compared to dilution, covers, and inoculum removal. It highlights acidification as a promising farm practice, although more testing is needed prior to on-farm implementation. Manure TS and VS concentrations had a good correlation with total CH_4 production, which supports previous research. However, our results

also suggest that only large differences in TS and VS could reduce CH_4 production meaningfully. Therefore, treatments focusing on TS and VS reduction may be unrealistic without large infrastructure for complete solid-liquid separation.

2.3. Future Research

Future research should continue to expand upon this work to go beyond the limitations of this research. Throughout all trials, GHG monitoring ended prior to tank emptying and manure field spreading. However, there may be extra CH_4 off-gassing during tank emptying and NH_3 volatilization during manure spreading. Additionally, the nitrogen cycle will continue to produce NO_3^- and N_2O and there may be unique effects of manure strategies during and following manure spreading. Other downstream effects may also warrant investigation, such as remnant sulphur compounds, nutrient and mineral composition, or changes in soil microorganismal growth. Some research has already addressed these topics, though it is very limited and still unclear (Eriksen et al. 2008; Lin et al. 2020).

2.3.1. Inoculum

Removal of residual manure in storage tanks has been tested in 3 trials at BEEC. Given the limitations of the meso-scale research, the next step should be testing the applicability of tank cleaning in a farm setting. The large size of on-farm manure storages will pose new challenges to tank filling and new cleaning techniques may need to be explored. Although inoculum removal has shown good GHG mitigation, it may be unfeasible if the inoculum removal process is too costly or difficult for farmers.

2.3.2. Acidification

Acidification with H_2SO_4 has shown exceptional GHG reductions, even at low concentrations. More research, however, may be necessary to uncover the mechanism by which H_2SO_4 disrupts methanogens. The sulfate present may be disrupting the methanogens, rather than the low pH.

An additional laboratory study with different acids and sulfate levels may help to find a better compound for reducing GHGs, which is safer for use than H_2SO_4. Additionally, given that H_2SO_4 may emit sulfur-based compounds following acidification, future research should monitor concentrations of sulfur emissions. These have the potential to be harmful to humans or farm animals at high concentrations. Given that H_2SO_4 is a dangerous substance, it is important to test any possible human hazards prior to on-farm testing. Lastly, given the bias shown in batch-filling with inoculum, there may be a similar bias in the acidification trials which were all filled in batch. Future research should look at the effect of gradual filling on the treatment effectiveness of acidification.

2.4. Recommended strategies for reduction GHG emissions

- Fully removing inoculating residual manure from storage tanks can reduce emissions by up to 50%, depending on the amount usually remaining after emptying.
- The amount of inoculating residual manure removed will reduce GHG emissions correspondingly, therefore any improvement in tanks cleaning will help.
- Acidifying residual manure with H_2SO_4 in lieu of removal will neutralize the inoculating effect and reduce emissions.
- Acidifying fresh manure with H_2SO_4 can reduce emissions by up to 89%, depending on the application rate.
- Acidifying fresh manure every other storage period will reduce emissions in the second period by ~30%.
- Cost of acidification may be off set by carbon credits earned due to GHG mitigation.
- Diluting manure may reduce GHG emissions up to 50%, although this may be off set by the larger volume of manure.

- Synthetic Biocap© covers did not show any reduction of CH_4, which is the predominant GHG gas from liquid dairy manure, therefore it is not recommended for GHG mitigation.
- Straw covers reduced CH_4 emissions by 20%, NH_3 by 70%, and N_2O by 60%. Although the GHG reductions were not large, the easy of use makes straw covering a very good mitigating practice.

Appendix

Image 1 Dalhousie University Bio-Environmental Engineering Centre experimental research site, showing instrument trailer (A) housing trace gas analysers, in-ground meso-scale manure with continuously flow-through, steady state chambers (B), and an instrument hut housing ammonia acid traps (C).

Image 2 Front view of in-ground, meso-scale manure tanks with continuously flow-through, steady-state chamber. Showing ambient air inflow vents.

Image 3 Rear view of in-ground, meso-scale manure tanks with continuously flow-through, steady-state chambers attached. Showing the outflow vent with sample tubing.

Image 4 Experimental manure tank during tank filling showing 80 cm depth manure thermocouples (A) and 10 cm above manure surface shielded air temperature thermocouples (B).

Image 5 Aluminium pole with acid resistant tubing taped to it (A) used for transferring sulphuric acid (H_2SO_4) throughout the stored manure and a peristaltic pump (B).

Bibliography

Agriculture and Agri-Food Canada (2016) An Overview of the Canadian Agriculture and Agri-Food System 2016. http://www.agr.gc.ca/eng/about-us/publications/economic-publications/an-overview-of-the-canadian-agriculture-and-agri-food-system-2016/?id=1462288050282

Agriculture and Agri-Food Canada (1980) Canada animal manure management guide

Agriculture and Agri-Food Canada, Canadian Dairy Information Centre (2019) Number of Dairy Cows and Heifers by Province

Alexander M (1977) Introduction to soil microbiology, 2nd edn. John Wiley & Sons, Inc, Toronto

American Public Health Association, Water Environmental Federation (2005) Standard methods for the examination of water and wastewater. Am Public Health Assoc APHA Wash DC USA

Apelt M, Benndorf R, Fischer PU, et al (2016) Collection of Measurement Methods for Biogas – Methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector. Deutsches Biomasseforschungszentrum gemeinnutzige GmbH, Leipzig

Baldé H, VanderZaag AC, Burtt S, et al (2016a) Measured versus modeled methane emissions from separated liquid dairy manure show large model underestimates. Agric Ecosyst Environ 230:261–270. https://doi.org/10.1016/j.agee.2016.06.016

Baldé H, VanderZaag AC, Burtt SD, et al (2016b) Does Fall Removal of the Dairy Manure Sludge in a Storage Tank Reduce Subsequent Methane Emissions? J Environ Qual 45:2038–2043. https://doi.org/10.2134/jeq2016.03.0083

Baral KR, Jégo G, Amon B, et al (2018) Greenhouse gas emissions during storage of manure and digestates: Key role of methane for prediction and mitigation. Agric Syst 166:26–35. https://doi.org/10.1016/j.agsy.2018.07.009

Berg W, Brunsch R, Pazsiczki I (2006a) Greenhouse gas emissions from covered slurry compared with uncovered during storage. Agric Ecosyst Environ 112:129–134. https://doi.org/10.1016/j.agee.2005.08.031

Berg W, Pazsiczki I (2006) Mitigation of methane emissions during manure storage. Int Congr Ser 1293:213–216. https://doi.org/10.1016/j.ics.2006.02.050

Berg W, Türk M, Hellebrand HJ (2006b) Effects of acidifying liquid cattle manure with nitric or lactic acid on gaseous emissions. In: Proceedings Workshop on Agricultural Air Quality: State of the Science. pp 492–498

Blake LI, Tveit A, Øvreås L, et al (2015) Response of Methanogens in Arctic Sediments to Temperature and Methanogenic Substrate Availability. PLOS ONE 10:e0129733. https://doi.org/10.1371/journal.pone.0129733

Borst GH (2001) [Acute poisoning of pigs with hydrogen sulfide as a result of acidification of slurry on a pig farm]. Tijdschr Diergeneeskd 126:104–105

Bothe H, Ferguson SJ, Newton WE (eds) (2007) Biology of the nitrogen cycle. Elsevier, Amsterdam

British Columbia Ministry of Agriculture (2015) Sizing dairy manure storage structures. British Columbia Ministry of Agriculture

Browne JD, Allen E, Murphy JD (2013) Evaluation of the biomethane potential from multiple waste streams for a proposed community scale anaerobic digester. Environ Technol 34:2027–2038. https://doi.org/10.1080/09593330.2013.812669

Burke D (2001) Dairy Waste Anaerobic Digestion Handbook. Environmental Energy Company, Olympia

Canadian Dairy Information Centre (2017) Canadian Dairy Industry at a Glance - Canadian Dairy Information Centre (CDIC). http://www.dairyinfo.gc.ca/index_e.php?s1=cdi-ilc&s2=aag-ail

Clemens J, Bergmann S, Vandré R (2002) Reduced Ammonia Emissions from Slurry after Self-Acidification with Organic Supplements. Environ Technol 23:429–435. https://doi.org/10.1080/09593332508618400

Clesceri LS, Greenber AE, Eaton AD (1998) Standard methods for the examination of water and waste water, 20th edn. American Public Health Association, American Water Works association, Water Environment Federation, Washington, DC

Dewes T (1996) Effect of pH, temperature, amount of litter and storage density on ammonia emissions from stable manure. J Agric Sci 127:501–509. https://doi.org/10.1017/S0021859600078722

Dong H, Mangino J, McAllister T, et al (2006) Chapter 10 Emissions from livestock and manure management. In: 2006 IPCC guidelines for national greenhouse gas inventories. IPCC, p 10.1-10.87

Environment and Climate Change Canada (2017a) National Inventory Report 1990-2015: Greenhouse gas sources and sinks in Canada

Environment and Climate Change Canada (2012) Progress towards Canada's greenhouse gas emissions reduction target. In: aem. https://www.canada.ca/en/environment-climate-change/services/environmental-indicators/progress-towards-canada-greenhouse-gas-emissions-reduction-target.html

Environment and Climate Change Canada (2011) Hourly Data Report for June 30, 2017 - Climate - Environment and Climate Change Canada. http://climate.weather.gc.ca/climate_data/hourly_data_e.html?hlyRange=2003-12-11%7C2018-06-28&dlyRange=2003-12-11%7C2018-06-27&mlyRange=2004-01-01%7C2007-07-01&StationID=42243&Prov=NS&urlExtension=_e.html&searchType=stnName&optLimit=yearRange&StartYear=1840&EndYear=2018&selRowPerPage=25&Line=1&searchMethod=contains&Month=6&Day=30&txtStationName=debert&timeframe=1&Year=2017

Environment and Climate Change Canada (2017b) Technical paper on the federal carbon pricing backstop. Environment and Climate Change Canada, Gatineau, QC

Eriksen J, Sørensen P, Elsgaard L (2008) The Fate of Sulfate in Acidified Pig Slurry during Storage and Following Application to Cropped Soil. J Environ Qual 37:280–286. https://doi.org/10.2134/jeq2007.0317

Fangueiro D, Hjorth M, Gioelli F (2015) Acidification of animal slurry– a review. J Environ Manage 149:46–56. https://doi.org/10.1016/j.jenvman.2014.10.001

Fangueiro D, Ribeiro H, Coutinho J, et al (2010) Nitrogen mineralization and CO2 and N2O emissions in a sandy soil amended with original or acidified pig slurries or with the relative fractions. Biol Fertil Soils 46:383–391. https://doi.org/10.1007/s00374-010-0444-1

Fangueiro D, Ribeiro H, Vasconcelos E, et al (2009) Treatment by acidification followed by solid–liquid separation affects slurry and slurry fractions composition and their potential of N mineralization. Bioresour Technol 100:4914–4917. https://doi.org/10.1016/j.biortech.2009.04.032

Fangueiro D, Surgy S, Coutinho J, Vasconcelos E (2013) Impact of cattle slurry acidification on carbon and nitrogen dynamics during storage and after soil incorporation. J Plant Nutr Soil Sci 176:540–550. https://doi.org/10.1002/jpln.201200117

Ferry JG (2010) The chemical biology of methanogenesis. Planet Space Sci 58:1775–1783. https://doi.org/10.1016/j.pss.2010.08.014

Fillingham MA, VanderZaag AC, Burtt S, et al (2017) Greenhouse gas and ammonia emissions from production of compost bedding on a dairy farm. Waste Manag 70:45–52. https://doi.org/10.1016/j.wasman.2017.09.013

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N2O production and consumption in soil. Exch Trace Gases Terr Ecosyst Atmosphere 47:7–21

Government of Canada E and CCC (2012) Environment and Climate Change Canada - Environmental Indicators - Global greenhouse gas emissions. http://www.ec.gc.ca/indicateurs-indicators/default.asp?lang=en&n=54C061B5-1. Accessed 4 Jul 2017

Guest G, Smith W, Grant B, et al (2017) A comparative life cycle assessment highlighting the trade-offs of a liquid manure separator-composter in a Canadian dairy farm system. J Clean Prod 143:824–835. https://doi.org/10.1016/j.jclepro.2016.12.041

Habtewold J, Gordon R, Sokolov V, et al (2018a) Targeting Bacteria and Methanogens To Understand the Role of Residual Slurry as an Inoculant in Stored Liquid Dairy Manure. Appl Environ Microbiol 84:. https://doi.org/10.1128/AEM.02830-17

Habtewold J, Gordon R, Sokolov V, et al (2018b) Reduction in Methane Emissions From Acidified Dairy Slurry Is Related to Inhibition of Methanosarcina Species. Front Microbiol 9:. https://doi.org/10.3389/fmicb.2018.02806

Hofer S (2003) Determination of ammonia (Salicylate) in 2 M KCl soil extracts by flow injection analysis. Lachat Instruments, Loveland, Colorado

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland

Janzen HH (2008) Better Farming, Better Air: A Scientific Analysis of Farming Practice and Greenhouse Gases in Canada. Agriculture and Agri-Food Canada

Jayasundara S, Ranga Niroshan Appuhamy JAD, Kebreab E, Wagner-Riddle C (2016) Methane and nitrous oxide emissions from Canadian dairy farms and mitigation options: An updated review. Can J Anim Sci 96:306–331. https://doi.org/10.1139/cjas-2015-0111

Kafle GK, Kim SH (2013) Anaerobic treatment of apple waste with swine manure for biogas production: Batch and continuous operation. Appl Energy 103:61–72. https://doi.org/10.1016/j.apenergy.2012.10.018

Kai P, Pedersen P, Jensen JE, et al (2008) A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. Eur J Agron 28:148–154. https://doi.org/10.1016/j.eja.2007.06.004

Kavanagh I, Burchill W, Healy MG, et al (2019) Mitigation of ammonia and greenhouse gas emissions from stored cattle slurry using acidifiers and chemical amendments. J Clean Prod 237:117822. https://doi.org/10.1016/j.jclepro.2019.117822

Kindaichi T, Awata T, Mugimoto Y, et al (2016) Effects of organic matter in livestock manure digester liquid on microbial community structure and in situ activity of anammox granules. Chemosphere 159:300–307. https://doi.org/10.1016/j.chemosphere.2016.06.018

Krupa SV (2003) Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review. Environ Pollut 124:179–221. https://doi.org/10.1016/S0269-7491(02)00434-7

Le Riche EL, Vanderzaag A, Wagner-Riddle C, et al (2017) Do volatile solids from bedding materials increase greenhouse gas emissions for stored dairy manure? Can J Soil Sci

Le Riche EL, VanderZaag AC, Wood JD, et al (2016) Greenhouse Gas Emissions from Stored Dairy Slurry from Multiple Farms. J Environ Qual 45:1822. https://doi.org/10.2134/jeq2016.04.0122

Le Riche EL, VanderZaag AC, Wood JD, et al (2020) Does overwintering change the inoculum effect on methane emissions from stored liquid manure? J Environ Qual 49:247–255. https://doi.org/10.1002/jeq2.20003

Lefcourt AM, Meisinger JJ (2001) Effect of adding alum or zeolite to dairy slurry on ammonia volatilization and chemical composition. J Dairy Sci 84:1814–1821

Lin H, Sun W, Yu Q, Ma J (2020) Acidic conditions enhance the removal of sulfonamide antibiotics and antibiotic resistance determinants in swine manure. Environ Pollut 263:114439. https://doi.org/10.1016/j.envpol.2020.114439

Livingston GP, Hutchinson GL (1995) Enclosure-based measurement of trace gas exchange: applications and sources of error. Biog Trace Gases Meas Emiss Soil Water 51:14–51

Lyberatos G, Skiadas IV (1999) Modelling of anaerobic digestion–a review. Glob Nest Int J 1:63–76

Mao C, Feng Y, Wang X, Ren G (2015) Review on research achievements of biogas from anaerobic digestion. Renew Sustain Energy Rev 45:540–555. https://doi.org/10.1016/j.rser.2015.02.032

Massé DI, Croteau F, Patni NK, Masse L (2003) Methane Emissions from Dairy Cow and Swine Manure Slurries Stored at 10°C and 15°C. Can Biosyst Eng 45:6–1

Massé DI, Jarret G, Hassanat F, et al (2016) Effect of increasing levels of corn silage in an alfalfa-based dairy cow diet and of manure management practices on manure fugitive methane emissions. Agric Ecosyst Environ 221:109–114. https://doi.org/10.1016/j.agee.2016.01.018

Massé DI, Masse L, Claveau S, et al (2008) Methane emissions from manure storages. Trans ASABE 51:1775–1781

Møller HB, Sommer SG, Ahring BK (2004a) Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenergy 26:485–495. https://doi.org/10.1016/j.biombioe.2003.08.008

Møller HB, Sommer SG, Ahring BK (2004b) Biological Degradation and Greenhouse Gas Emissions during Pre-Storage of Liquid Animal Manure. Methane Product Nutr Recovery Manure 74:496

Monod J (1949) The Growth of Bacterial Cultures. Annu Rev Microbiol 3:371–394. https://doi.org/10.1146/annurev.mi.03.100149.002103

Nges IA, Liu J (2010) Effects of solid retention time on anaerobic digestion of dewatered-sewage sludge in mesophilic and thermophilic conditions. Renew Energy 35:2200–2206. https://doi.org/10.1016/j.renene.2010.02.022

Ngwabie NM, Gordon RJ, VanderZaag A, et al (2016) The Extent of Manure Removal from Storages and Its Impact on Gaseous Emissions. J Environ Qual 45:2023–2029. https://doi.org/10.2134/jeq2016.01.0004

Nyord T, Liu D, Eriksen J, Adamsen APS (2013) Effect of acidification and soil injection of animal slurry on ammonia and odour emission. In: Proceeding of the 14th RAMIRAN International Conference in Versailles, France. http://www. ramiran. net/doc13/Proceeding_2013/homepage. html

Owen JJ, Silver WL (2015) Greenhouse gas emissions from dairy manure management: a review of field-based studies. Glob Change Biol 21:550–565. https://doi.org/10.1111/gcb.12687

Pachauri RK, Mayer L (eds) (2015) Climate change 2014: synthesis report. Intergovernmental Panel on Climate Change, Geneva, Switzerland

Petersen SO, Amon B, Gattinger A (2005) Methane oxidation in slurry storage surface crusts. J Environ Qual 34:455–461

Petersen SO, Andersen AJ, Eriksen J (2012) Effects of Cattle Slurry Acidification on Ammonia and Methane Evolution during Storage. J Environ Qual 41:88. https://doi.org/10.2134/jeq2011.0184

Petersen SO, Hutchings NJ, Hafner SD, et al (2016) Ammonia abatement by slurry acidification: A pilot-scale study of three finishing pig production periods. Agric Ecosyst Environ 216:258–268. https://doi.org/10.1016/j.agee.2015.09.042

Regueiro I, Coutinho J, Fangueiro D (2016) Alternatives to sulfuric acid for slurry acidification: impact on slurry composition and ammonia emissions during storage. J Clean Prod 131:296–307. https://doi.org/10.1016/j.jclepro.2016.05.032

Rochette P, Hutchinson GL (2005) Measurement of soil respiration in situ: chamber techniques

Schils RLM, Meer HG van der, Wouters AP, et al (1999) Nitrogen utilization from diluted and undiluted nitric acid treated cattle slurry following surface application to grassland. Nutr Cycl Agroecosystems 53:269–280. https://doi.org/10.1023/A:1009745204647

Sheppard S, Bittman S, Swift M, et al (2011) Ecoregion and farm size differences in dairy feed and manure nitrogen management: A survey. Can J Anim Sci 91:459–473. https://doi.org/10.4141/cjas2010-004

Shi Y, Parker DB, Cole NA, et al (2001) Surface amendments to minimize ammonia emissions from beef cattle feedlots. Trans ASAE 44:677

Shin S-R, Im S, Mostafa A, et al (2019) Effects of Pig Slurry Acidification on Methane Emissions during Storage and Subsequent Biogas Production. Water Res. https://doi.org/10.1016/j.watres.2019.01.005

Sokolov V, VanderZaag A, Habtewold J, et al (2019a) Greenhouse gas emissions from gradually-filled liquid dairy manure storages with different levels of inoculant. Nutr Cycl Agroecosystems 115:455–467. https://doi.org/10.1007/s10705-019-10023-2

Sokolov V, VanderZaag A, Habtewold J, et al (2019b) Greenhouse Gas Mitigation through Dairy Manure Acidification. J Environ Qual 0: https://doi.org/10.2134/jeq2018.10.0355

Sokolov VK, VanderZaag A, Habtewold J, et al (2020) Dairy manure acidification reduces CH4 emissions over short and long-term. Environ Technol 1–8. https://doi.org/10.1080/09593330.2020.1714744

Sommer SG, Christensen ML, Schmidt T, Jensen LS (2013) Animal manure recycling: Treatment and management. Wiley, West Sussex

Sommer SG, Petersen SO, Sorensen P, et al (2007) Methane and carbon dioxide emissions and nitrogen turnover during liquid manure storage. Nutr Cycl Agroecosystems 78:27–36. https://doi.org/10.1007/s10705-006-9072-4

Sørensen P, Eriksen J (2009) Effects of slurry acidification with sulphuric acid combined with aeration on the turnover and plant availability of nitrogen. Agric Ecosyst Environ 131:240–246. https://doi.org/10.1016/j.agee.2009.01.024

Statistics Canada (2014) Highlights. http://www.statcan.gc.ca/pub/91-520-x/2014001/hi-fs-eng.htm

United Nations Department of Economic and Social Affairs (2017) World population prospects: The 2017 revision. United Nations, New York

Van der Stelt B, Temminghoff EJM, Van Vliet PCJ, Van Riemsdijk WH (2007) Volatilization of ammonia from manure as affected by manure additives, temperature and mixing. Bioresour Technol 98:3449–3455. https://doi.org/10.1016/j.biortech.2006.11.004

VanderZaag AC (2010) Measurement and mitigation of gaseous emissions from dairy wastewater treatment and storage systems. Library and Archives Canada = Bibliothèque et Archives Canada

VanderZaag AC, Baldé H, Crolla A, et al (2017) Potential methane emission reductions for two manure treatment technologies. Environ Technol 1–8. https://doi.org/10.1080/09593330.2017.1313317

VanderZaag AC, Gordon RJ, Jamieson RC, et al (2010a) Effects of winter storage conditions and subsequent agitation on gaseous emissions from liquid dairy manure. Can J Soil Sci 90:229–239

VanderZaag AC, Gordon RJ, Jamieson RC, et al (2009) Gas emissions from straw covered liquid dairy manure during summer storage and autumn agitation. Trans ASABE 52:599–608

VanderZaag AC, Gordon RJ, Jamieson RC, et al (2010b) Permeable synthetic covers for controlling emissions from liquid dairy manure. Appl Eng Agric 26:287–297

Vavilin VA, Fernandez B, Palatsi J, Flotats X (2008) Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. Waste Manag 28:939–951. https://doi.org/10.1016/j.wasman.2007.03.028

Vedrenne F, Béline F, Dabert P, Bernet N (2008) The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. Bioresour Technol 99:146–155. https://doi.org/10.1016/j.biortech.2006.11.043

Wang K, Huang D, Ying H, Luo H (2014) Effects of acidification during storage on emissions of methane, ammonia, and hydrogen sulfide from digested pig slurry. Biosyst Eng 122:23–30. https://doi.org/10.1016/j.biosystemseng.2014.03.002

Wood JD, Gordon RJ, Wagner-Riddle C, et al (2012) Relationships between Dairy Slurry Total Solids, Gas Emissions, and Surface Crusts. J Environ Qual 41:694. https://doi.org/10.2134/jeq2011.0333

Wood JD, Gordon RJ, Wagner-Riddle C (2013) Biases in discrete CH4 and N2O sampling protocols associated with temporal variation of gas fluxes from manure storage systems. Agric For Meteorol 171–172:295–305. https://doi.org/10.1016/j.agrformet.2012.12.014

Wood JD, VanderZaag AC, Wagner-Riddle C, et al (2014a) Gas emissions from liquid dairy manure: complete versus partial storage emptying. Nutr Cycl Agroecosystems 99:95–105. https://doi.org/10.1007/s10705-014-9620-2

Wu Y, Gu B, Erisman JW, et al (2016) PM 2.5 pollution is substantially affected by ammonia emissions in China. Environ Pollut 218:86–94. https://doi.org/10.1016/j.envpol.2016.08.027

Zeeman G (1994) Methane production/emission in storages for animal manure. Nutr Cycl Agroecosystems 37:207–211