# Application of manure from cattle fed with different diets affects soil properties and greenhouse gas emissions

by

Tien Luo Ying Weber

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Department of Renewable Resources
University of Alberta

## Abstract

Cattle production contributes the largest portion of livestock agricultural greenhouse gas (GHG) emissions in Canada mostly in the form of methane ($CH_4$). Efforts to reduce $CH_4$ emissions have led to modification of diet composition for livestock, resulting in a corresponding change in manure properties. The release of nitrous oxide ($N_2O$) and $CH_4$ from manure is the second leading cause of GHG emissions in agriculture. However, the effect of diet modification on GHG emission from cattle manure has not been well studied. Therefore, I designed two laboratory incubation experiments to investigate how amending the soil with manure from cattle fed different diets affect GHG emissions and soil biogeochemical properties. Total nitrogen (N), total carbon (C), organic C (OC), C/N ratio, microbial biomass C, microbial biomass N, nitrate ($NO_3^-$-N), ammonium ($NH_4^+$-N), available N (sum of $NO_3^-$-N and $NH_4^+$-N) and GHG fluxes (carbon dioxide ($CO_2$), $N_2O$, and $CH_4$) were measured throughout the experiments to understand the C and N cycles.

The first incubation was conducted over 70 days. Manure from cattle fed a corn-based silage diet (CM) or a barley-based silage diet (BM) was applied to three soil types (collected from the upper 0-15 cm): Orthic Black Chernozem (OBC), Dark Brown Chernozem (DBC), and Orthic Gray Luvisol (OGL). Those treatments were compared with a control (CK) without manure application. The $CO_2$, $N_2O$, and $CH_4$ emissions from the CK soil were lowest while amended soils amended had higher GHG emissions because the solubility of OC was increased by the addition of manure. The $CO_2$ emissions were greatest in CM amendments across all soil type, likely resulting from a difference in C quality or another variable that was not investigated in this study. The $N_2O$ emissions were found to be greatest in BM amended OBC and OGL soils due to the variation in fecal and urinary forms of N. The $CH_4$ emissions were highest from BM-amended soils in OBC and OGL soil types from the difference in location of starch digestion in the cattle's rumen which may have altered the properties of the BM and CM amendments.

The second incubation was conducted over 85 days. The experiment used (0-15 cm) soil of neutral fine-textured Orthic Dark Brown Chernozem (DBC), an acidic fine-textured Orthic Gray Luvisol (OGL), and an acidic coarse-textured Orthic Black Chernozem (OBC) amended with manure from cattle fed a barley-based diet (BM), a barley-based diet supplemented with the methane inhibitor 3-nitrooxypropanol (3-NOP; 3NOPM), or composted 3NOPM (3NOPC). Those treatments were compared with a control (CK) without manure application. The BM had the lowest nitrate ($NO_3^-$-N) concentration and higher C/N ratio and the BM-amended soil had the highest $CO_2$ emissions in the OBC and OGL amended soils. The 3NOPC resulted in lower $N_2O$ emissions than 3NOPM across all soil types from stabilized C and N that limited microbial activity. The OGL soil had the highest $CO_2$, $N_2O$, and $CH_4$ flux from the low initial pH that stimulated labile C consumption. The positive $CH_4$ emissions from OGL and DBC clay loam soils comes from the ability to retain water and promote methanogen transport.

In conclusion, our results from the first experiment found that manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected GHG emissions, which were the greatest for BM-amended and least for CK soils. Therefore, feeding cattle corn- rather than barley-based diet may be more environmentally friendly, specifically if the manure is applied to coarse-textured soils. Our results from the second experiment show that GHG emissions for composted manure from cattle fed 3-NOP were the lowest, indicating that 3-NOP supplementation of cattle diets has the potential to reduce total GHG emissions, especially in neutral soils. Further research is needed to investigate the effect on GHG emissions of manure application from cattle fed various diets in the field and in other soil types.

## Preface

This dissertation is an original work conducted by Tien Luo Ying Weber. Versions of chapters 2 and 3 are being prepared for submission to peer-reviewed journals. I am responsible for writing chapters 1 and 4, and for data collection, analysis, and manuscript writing for chapter 2 and 3. Chang, S. and Hao, X. were the supervisory authors and they were involved in research concept formulation and edited manuscripts. K.A. Beauchemin, C.D. Gross, and D. Kaliaskar were the coauthors and they were involved in editing manuscripts.

## Dedication

To my parents, Michele and David Weber, and to everyone who studies soils.

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## Chapter 1. General Introduction

### 1.1 The Greenhouse Gases and the Greenhouse Gas Effect

The anthropogenic impact from all aspects has created a strain on Earth's natural resources, from the direct use of fossil fuels to the indirect impact of pollution. One such example is the greenhouse effect, defined as the "*presence of certain gases which trap long-wave radiation emitted from the earth surface*" that results in warming of the earth's surface (Mitchell, 1989). The primary greenhouse gases (GHG) responsible for the greenhouse effect are carbon dioxide ($CO_2$), nitrous oxide ($N_2O$), and methane ($CH_4$) (Boadi et al., 2004; Liebig et al., 2005; Smith et al., 2008). Different gases contribute to the greenhouse effect at varying degrees, for example, $N_2O$ and $CH_4$ have absorption bands near 9.6 $\mu$m, meaning that they can trap long-wave radiation despite low concentrations (Mitchell, 1989).

While anthropogenic impacts are now the primary source of global warming, this wasn't always the case. The majority of greenhouse gases occur naturally, and without human intervention, the concentration of these gases can either rise or fall (Mitchell, 1989). However, since the Industrial Revolution in the middle of the 19th century, the GHG concentrations have increased due to human impact (Mitchell, 1989). The initial increase of $CO_2$ since has occurred due to the burning of fossil fuels (Mitchell, 1989; Boadi et al., 2004).

The $N_2O$ emissions are produced through both nitrification (ammonium ($NH_4^+$) and oxygen ($O_2$) react to form $N_2O$) and denitrification (bacteria reduce $NO_3^-$ to $N_2O$ or $N_2$ due to reduced soil redox potential) (Weier et al., 1993; Liebig et al., 2005; Sampanpanish, 2012; Thangarajan et al., 2013). Denitrification results in higher $N_2O$ emissions than nitrification (Thangarajan et al., 2013). Processes such as microbial interactions in soil and water, production of fertilizers and fossil fuels are responsible for increased $N_2O$ concentrations that are heating the troposphere (Mitchell, 1989).

Methane is not only one of the most abundant organic trace gases but also has an infrared absorbance 20-30 times greater than $CO_2$ when re-emitted to the Earth's surface (Thangarajan et al., 2013). Greenhouse gas emissions from paddy fields, ruminants, and wetlands are the primary sources of $CH_4$ emissions (Mitchell, 1989; Smith et al., 2008). Similar to $CO_2$ and $N_2O$, $CH_4$ emissions are produced by organic decomposition by microbial activity; however, unlike the other gases, it is produced in anaerobic environments in a process called methanogenesis (Eckard et al., 2010; Sampanpanish, 2012; Thangarajan et al., 2013).

## 1.2 Greenhouse Gas Emissions from Agriculture

Approximately 10% of anthropogenic GHG emission in Canada is attributed to agricultural activities, with livestock production contributing approximately 42% of these agricultural emissions, and around 28% of livestock emissions are from enteric fermentation (Boadi et al., 2004; Smith et al., 2008; Jayasundara et al., 2016). Multiple agricultural practices have resulted in a 41% increase in GHG emissions from Canadian beef production since 1990 (Beauchemin et al., 2010).

Carbon dioxide emissions from agricultural practices result as the end product of microbial respiration (Liebig et al., 2005; Smith et al., 2008). Agricultural practices can also act as a $CO_2$ sink, as soil can sequester C and reduce atmospheric $CO_2$ (Liebig et al., 2005). Farm fieldwork and the use of supply machinery can also contribute to $CO_2$ emissions (Vergé et al., 2008). Fortunately, a decline in the use of fossil fuels has helped decrease $CO_2$ emissions (Vergé et al., 2008).

Agricultural activity is responsible for 60% of all anthropogenic $N_2O$ produced (Pattey et al., 2005; Eckard et al., 2010). Microbial transformation of N in soils into $N_2O$ emissions can increase when available N exceeds plant requirements (Smith et al., 2008). Over-application of N fertilizers has been a source of $N_2O$ emissions, which has been declining as more sustainable practices are being introduced (Vergé et al., 2008).

Agriculture contributes 50% of all anthropogenic $CH_4$ emissions (Pattey et al., 2005; Eckard et al., 2010). Additionally, production of enteric $CH_4$ emissions from cattle can be a significant loss of dietary energy (3%-12%), meaning an economic loss for farmers (Vyas et al., 2018). Soils in anaerobic environments serve as a $CH_4$ source from the reduction of $CO_2$ and soils in aerobic conditions serve as a $CH_4$ sink from the oxidation of $CH_4$ (Liebig et al., 2005). Methane emissions from the cattle industry are primarily from enteric fermentation and manure storage (Vergé et al., 2008). Energy loss from enteric $CH_4$ can range from 2 to 12% of feed intake (Beauchemin and McGinn, 2005). There has been an increase in $CH_4$ emissions over the years due to an increase in the weight of individual Canadian beef cattle (Vergé et al., 2008).

A major contributor to $CH_4$ emissions is the microbial fermentation of livestock feed components (Beauchemin and McGinn, 2005; Beauchemin et al., 2010). In the rumen, methanogens commonly use $H_2$, $CO_2$, and formate as substrates in methanogenesis (Johnson et al., 2000; Boadi et al., 2004). Methanogens are involved in the final stages of fermentation and are important in utilizing electrons in the form of $H_2$, which lowers $H_2$ pressure in the rumen and increase digestion (Boadi et al., 2004). As $CH_4$ is nothing but a loss of energy to the cattle, it is emitted and contributes to GHG emissions (Boadi et al., 2004).

Agricultural practices have the potential to mitigate the effects of climate change by increasing soil organic carbon, better understanding ways to decrease $CH_4$ and $N_2O$ emissions, and increasing $CH_4$ soil oxidation (Smith et al., 2008). To correctly assess GHG mitigation strategies, one must conduct a life cycle assessment (LCA) that encompasses the entire farming system as reducing GHG in one part can increase GHG in another part of the process (Beauchemin et al., 2010). For example, the LCA begins when the breeding stock are born, continues through meat production cycles which include the release of manure, and ends with the birth of replacements and slaughter of breeding stock (Beauchemin et al., 2010).

Some examples of ways to mitigate GHG emissions from agricultural practices include using improved crop varieties, extending crop rotations, avoiding bare fallow, adding more nutrients when necessary, reducing tillage, restoration of degraded agricultural lands, and adding shelter belts (Smith et al., 2008). Efforts to mitigate GHG emissions from livestock include decreasing grazing intensity, switching to bioenergy, improving manure management, and altering livestock diets (Smith et al., 2008). This research focuses on the last two GHG emission mitigation strategies.

### 1.3 Livestock Diet Manipulation

The focus of the two experiments was on how different feed compositions and additives to cattle diets can influence the later stages of LCA when the manure is applied to soil. Feed grains are the main source of protein in livestock diets (Spicer et al., 1986). Changing the feed composition of livestock can change the corresponding cattle manure properties, in hopes of reducing $CH_4$ emissions (Spicer et al., 1986; Beauchemin and McGinn, 2005; Eckard et al., 2010; Li et al., 2016b). A common practice is to feed a combination of grains for the best performance (Hunt, 1996; Johnson et al., 2000). Emissions will vary due to the amount of feed intake and diet composition (Beauchemin and McGinn, 2005).

For example, increasing feed levels increases the passage rate through the rumen, which reduces the time for dietary fermentation, and thus lowers $CH_4$ production (Johnson et al., 2000; Boadi et al., 2004). A common strategy used in Canada is aimed at improving animal productivity by increasing feed energy (Boadi et al., 2004). By increasing productivity per animal, the $CH_4$ emissions go up, but $CH_4$ emissions per unit of product decreases (Boadi et al., 2004). Not only are there environmental benefits, but the demand for low-fat milk is increasing, encouraging farmers to modify feeding regimes to use highly digestible protein feed which, in turn, is beneficial as it increases productivity (Boadi et al., 2004).

A traditional Canadian dairy feed consists of mostly barley silage (BS) in western Canada (Jayasundara et al., 2016). However, as western Canada faces dryer climates, the rising price of barley is encouraging farmers to look towards alternatives such as corn (Gibb and McAllister, 2003). Corn silage (CS) has greater starch than BS, and starch has been shown to reduce $CH_4$ emissions (Jayasundara et al., 2016). Therefore, by lowering the BS/CS ratio in cattle feed, there have been observed declines in enteric $CH_4$ emissions from dairy cattle (Jayasundara et al., 2016). This could be due to the shift from digestion in the rumen to the intestines for corn or the greater fermentability of the starch of barley (Johnson et al., 2000; Beauchemin and McGinn, 2005). Increasing the CS/BS ratio has also been found to increase total-tract digestibility of organic matter, resulting in lower C excretions (Jayasundara et al., 2016). However, dairy cows fed more corn silage excreted more organic matter in feces per unit of dry matter, so all factors must be considered when determining net anthropogenic GHG emissions (Jayasundara et al., 2016).

Cattle feed amendments may have the ability to improve feed digestibility, increasing protein and total organic matter which results in lower enteric $CH_4$ emissions (Jayasundara et al., 2016). Many amendments such as bromochloromethane and 2-bromo-ethane sulfonate have been shown to reduce $CH_4$ emissions without damaging repercussions, but their usage is limited due to potential toxicity, rumen adaption, or environmental regulations (Lopes et al., 2016). Ionophores, highly lipophilic substances that shield and delocalize the charge of ions, are also utilized (Johnson et al., 2000; Boadi et al., 2004). One example is Monensin (MON), commonly used in Canadian dairy feed to improve milk production, enhance antiketogenic effects, and reduce acidosis (Jayasundara et al., 2016). The MON is commonly used to improve the efficiency of feed utilization by shifting the fermentation from acetate to propionate production, inhibiting gram-positive bacteria (Boadi et al., 2004; Vyas et al., 2018). While MON has been shown to decrease $CH_4$ emissions by up to 25%, continued use of ionophores has not been shown to suppress $CH_4$ emissions due to antibiotic resistance, so other options must be considered (Johnson et al., 2000; Boadi et al., 2004; Beauchemin and McGinn, 2005; Jayasundara et al., 2016).

Another compound, three-nitrooxypropanol (3-NOP), is designed to inhibit the activity of methyl coenzyme-M reductase (MCR), the enzyme responsible for $CH_4$ formation (Lopes et al., 2016). Haisan (2014) found there was a 60% decline in emissions in cattle fed 2500 mg $d^{-1}$ of 3-NOP over 4 weeks. The effect of 3-NOP has been shown to decline two hours after feeding, suggesting that 3-NOP is absorbed, metabolized, and/or washed out from the rumen (Romero-Perez et al., 2014). One benefit is there are no observed effects on feed digestibility and low risk of food safety (Jayasundara et al., 2016; Vyas et al., 2018).

There have been varying results in 3-NOP success depending on the animal it is fed to, with no changes in $CH_4$ emissions observed in dairy cattle, but increases in $CH_4$ emissions for sheep (Romero-Perez et al., 2014). This variability in 3-NOP ability to lower $CH_4$ formation may be related to the composition of feed it has been added to (Haisan et al., 2014; Vyas et al., 2018). Additionally, the body mass index (BMI) of the livestock receiving the amendment has been shown to result in varying emissions as energy expenditure differs between different cattle and their lifestyles (Vergé et al., 2008). Lower $CH_4$ emissions are attributed to greater starch fermentation and ruminal propionate synthesis which reduces $H_2$ availability (Vyas et al., 2018). Therefore, high grain diets may reduce the abundance of MCR and $CH_4$ emissions (Vyas et al., 2018). The 3-NOP is still a relatively new product, and effects on manure, potential toxicology, and consumer acceptance are yet to be discovered (Jayasundara et al., 2016).

### 1.4 Greenhouse Gas Emissions from Manure-Amended Soils

Manure application as an organic amendment to crops is the second largest contributor to GHG emissions (Johnson et al., 2000; Jayasundara et al., 2016). Beauchemin et al. (2010) found that $N_2O$ and $CH_4$ emissions from manure accounted for 27% of total GHG emissions. Because large-scale operations often confine cattle, large amounts of dairy manure are accumulated in barns (Jayasundara et al., 2016). Proper agricultural management of this manure can mitigate the effects of GHG emissions (Liebig et al., 2005).

Cattle manure provides a beneficial organic amendment to the soil that enhances soil microbial activity (Rochette and Gregorich, 1998; Liebig et al., 2005; Thangarajan et al., 2013; Yang et al., 2015). Increased SOC from manure application is beneficial to crops as it increases nutrient cycling and improves soil physical and chemical properties (Miyittah and Inubushi, 2003; Chadwick et al., 2011; Zhang et al., 2015). This helps offset GHG emissions; however, the net GHG emission reduction or increase is determined by the type of manure applied and the rate of the application (Chadwick et al., 2011).

Application of excess manure can have deleterious effects on air and water quality (Liebig et al., 2005). For example, manure contains ammoniacal N, degradable C, and water, which can lead to excess $N_2O$ and $CH_4$ production when amended to the soil (Jayasundara et al., 2016). In addition, the increase in labile C pools from the manure induces microbial activity, producing greater rates of $CO_2$ gases (Gregorich et al., 1998; Calderón et al., 2004; Pattey et al., 2005; Sampanpanish, 2012). Manure application allows manure $NH_4^+$ to be transformed into $NO_3^-$ through nitrification (Chadwick et al., 2011). Manure high in $NH_4^+$ can also result in $NH_3$ volatilization which further contributes to GHG emissions when released into the atmosphere (Jayasundara et al., 2016).

The type of diet and diet additives have been linked to potential changes in manure properties (Chadwick et al., 2011; Jayasundara et al., 2016). For example, up to 40% of active MON has been found in feces, meaning that its antimethanogenic properties may still be active when applied to soils (Jayasundara et al., 2016). These changes to the manure could potentially reduce anthropogenic GHG emissions (Vergé et al., 2008; Chadwick et al., 2011). Likewise, the influence of different diet compositions may influence manure properties. Reducing the crude protein in diets can reduce N excretion, lowering $N_2O$ emissions (Chadwick et al., 2011). As corn silage is higher in energy and lower in protein than barley silage, it is hypothesized to reduce $N_2O$ emissions (Gibb and McAllister, 2003). In a similar way, I am interested in investigating the ability of 3-NOP to retain its antimethanogenic properties in manure. Also, I want to understand the differences in CS and BS when the respective manure is applied as an organic amendment to multiple soil types.

The type of soil the manure is applied to can also play a major role in the extent of GHG emissions. The soil texture can determine water holding capacity, which means potential waterlogging. In addition to choosing the appropriate manure management strategy given their soil type, drainage of agricultural lands can help productivity (and soil C) and potentially suppress $N_2O$ emissions (Smith et al., 2008). For example, clay soils had double the $N_2O$ emissions of sandy soils (Chadwick et al., 2011). The timing of manure application to the soil during the dry season could help reduce $CH_4$ emissions (Smith et al., 2008). Additionally, other soil properties such as a higher cation exchange capacity (CEC) can reduce N availability through increased adsorption of $NH_4^+$ in clay soils (Chadwick et al., 2011). For these reasons, I investigated different soil orders in our experiments to see potential interactions between different manure and soil types.

One particular agricultural management practice that is investigated in these experiments is composting, defined as an "*aerobic process that transforms biological waste materials into a stable humus-like material through microbial decomposition*" (Jayasundara et al., 2016). Approximately 38% of farms in Canada were using composting in 2001 (Jayasundara et al., 2016). There are two types of composting techniques: active, which forces aeration, or passive, where aeration is provided by the "chimney effect" (Jayasundara et al., 2016). Composting is beneficial as it reduces quantity, hauling costs, odor, and weed seeds in the manure (Schlegel, 1992). Adding composted manure to soil has been shown to reduce GHG emissions from 72-83% (Jayasundara et al., 2016). However, there is much variation in the overall success of composting, depending on the timing and frequency of turning the manure (Jayasundara et al., 2016).

The effect of diet on GHG emissions when applied as manure is only one part of the LCA (Chadwick et al., 2011). For example, the process of composting can release $CH_4$ and $N_2O$ from a microbial breakdown, even though applying the resulting compost reduces GHG emissions

(Jayasundara et al., 2016). Additionally, $N_2O$ emissions from barley production were <30% of corn, so the use of manure from cattle fed corn silage may not result in lower net GHG emissions (Liebig et al., 2005). Furthermore, the use of high grain diets will increase fossil fuel use and $N_2O$ emissions, so all aspects of the LCA must be considered (Boadi et al., 2004). In conclusion, the experiments conducted in my thesis only address small, but important, pieces of a bigger picture.

## 1.5 Research Question & Hypothesis

### *1.5.1 Chapter 2 research question & hypothesis*

The research question I set out to investigate was: *Will cattle manure from corn- vs barley-based diet influence GHG after soil application to different soil types?* Barley silage is higher in crude protein content (13% versus 8% of dry matter) than corn silage (Hunt, 1996; Gibb and McAllister, 2003; Hassanat et al., 2014; Chibisa and Beauchemin, 2018). Therefore, it was hypothesized that the GHG emissions will be lower in CM than BS and lower in sandy than clay and silt soils. This is because soil texture influences water-holding capacities which drive anaerobic and aerobic processes of $N_2O$ and $CH_4$ emissions.

The experiment in chapter 2 was conducted as a laboratory incubation. The manure treatments from a feedlot study conducted by Chibisa and Beauchemin (2018): (1) a corn-based silage diet (CM), a (2) barley-based silage diet (BM), or (3) no manure (CK, control) were applied to three soil types: (1) Orthic Black Chernozem (OBC), (2) Dark Brown Chernozem (DBC), and (3) Orthic Gray Luvisol (OGL). Total N, total C, organic C (OC), C/N ratio, microbial biomass C, microbial biomass N, nitrate ($NO_3^-$-N), ammonium ($NH_4^+$-N), available N (sum of $NO_3^-$-N and $NH_4^+$-N) and GHG fluxes ($CO_2$, $N_2O$, and $CH_4$) were measured to understand the C and N cycles.

### *1.5.2 Chapter 3 research question & hypotheses*

The research questions I set out to investigate were: *Will cattle manure from cattle fed diets supplemented with 3-nitrooxypropanol influence GHG after soil application to different soil types? What about composted cattle manure from cattle fed diets supplemented with 3-nitrooxypropanol?* A study by Lopes et al. (Lopes et al., 2016) found that 3-NOP diets reduced enteric $CH_4$ emissions by 31% as compared to the control. Another investigation by Lopes et al. (2016) found a similar result where 30% of $CH_4$ was reduced in a 12-week experiment. Therefore, due to the probability of 3-NOP being washed out of the rumen in the feces, it is hypothesized that soil amended with 3NOPM will have the lowest levels of anthropogenic greenhouse gas emission, with the regular diet manure having the highest levels of anthropogenic greenhouse gas emission (Romero-Perez et al., 2014).

Fresh manure consists of more than 80% water, resulting in large quantities of manure required for fertilization (Schlegel, 1992). Because of its liquid consistency, nitrate leaching is common. Composting manure brings water levels to 20-25% and stabilizes C and N, reducing the degradation rate and available N by 50% (Li et al., 2016a). Therefore, it is hypothesized that 3NOPC will result in lower greenhouse gas emissions than 3NOPM. Once again, because soil texture influences water holding capacity which drives anaerobic and aerobic processes of $N_2O$ and $CH_4$ emission, it was hypothesized that the anthropogenic GHG emissions will be lower in sandy than clay and silt soils.

The experiment in chapter 3 was also conducted as a laboratory incubation. The four manure treatments came from a feedlot study conducted by Vyas et al. (2018): soil amended with (1) stockpiled manure from cattle fed a typical feedlot diet (BM), (2) stockpiled manure from cattle fed a typical feedlot diet supplemented with 3-NOP (3NOPM), and (3) compost produced using manure in treatment (2) (3NOPC), and (4) a non-amended control (soil without manure) (CK). Each manure treatment was amended to each of the three soil types: (1) Dark Brown Chernozem (DBC), (2) Orthic Black Chernozem (OBC), and (3) Orthic Gray Luvisol (OGL). Total N, total C, OC, C/N ratio, nitrate ($NO_3^-$-N), ammonium ($NH_4^+$-N), available N (sum of $NO_3^-$-N and $NH_4^+$-N) and GHG fluxes ($CO_2$, $N_2O$, and $CH_4$) were measured to understand the C and N cycles.

# Chapter 2. Amending soil with manure from cattle fed corn-based diets reduces anthropogenic greenhouse gas emissions in an incubation experiment

## 2.1 Introduction

The livestock industry produces 42% of total greenhouse gas (GHG) production from agriculture (Boadi et al., 2004). Of this 42%, 28% is associated with enteric fermentation and 14% comes from manure handling, storage, and land application (Boadi et al., 2004). Cattle production is of particular interest, as it contributes to greater GHG emissions than any other livestock production system (de Vries and de Boer, 2010). The rumen of cattle is characterized by a diverse microbial community that carries out the necessary metabolic activities needed for feed digestion (van Vliet et al., 2007; Ozbayram et al., 2018). The number of nutrients present in the diet and the ability of cattle to digest and absorb the nutrients can vary among different grains, bringing interest in the manipulation of diet to reduce GHG emissions from livestock (Spicer et al., 1986; Guyader et al., 2018). Moreover, $N_2O$ and $CH_4$ emissions from cattle manure have increased GHG emissions from Canadian beef production by 41% since 1990 (Beauchemin et al., 2010). Because manure is often used as an amendment on soil, there is an additional need to evaluate the impact of manure produced from different diets on soil GHG emissions (Spicer et al., 1986; Beauchemin and McGinn, 2005; Eckard et al., 2010; Li et al., 2016b).

In recent years there has been a rapid expansion of corn silage on the Canadian prairies, presenting itself as a viable alternative in the cattle diet industry (Chibisa and Beauchemin, 2018; Guyader et al., 2018). In western Canada, the beef cattle industry uses mainly barley grain and barley silage components for backgrounding and finishing diets (Beauchemin and McGinn, 2005; Hassanat et al., 2014).  On average, barley silage is higher in crude protein content (13% versus 8% of dry matter) than corn silage, but 5-15% lower in digestible energy content and 30% lower in starch (Hunt, 1996; Gibb and McAllister, 2003; Hassanat et al., 2014; Chibisa and Beauchemin, 2018). These differences in digestible energy and nutrient content may affect the release of GHG emissions from the resulting manure.

Beauchemin and McGinn (2005) measured enteric methane ($CH_4$) emissions from cattle fed barley- versus corn-based diets and found that emissions were not affected by grain source during the backgrounding phase, but $CH_4$ emissions were lower for corn-fed cattle during the finishing phase. However, manure GHG emissions were not measured in these studies. There is a lack of studies on how diet composition affects cattle manure properties and GHG emissions and the influence of manure on soil properties and GHG emissions after it is applied as an organic amendment.

Improving our understanding of the impact of amending the soil with manure will help us to develop sustainable livestock management strategies (Gregorich et al., 1998; van Vliet et al., 2007). In order to remove external factors, such as soil temperature and water (which can alter the rate of soil organic matter degradation and biogeochemical cycle rates), I performed a laboratory incubation to test manure-amended soils (Rochette and Gregorich, 1998). The objectives of this experiment were to (i) determine the effect of manure type on soil carbon (C) and nitrogen (N) cycling from different soil types; (ii) determine the effect of manure type on GHG emissions from different soil types; and (iii) determine the relationship between C and N dynamics and GHG emissions.

## 2.2 Materials and Methods

### *2.2.1 The Experimental Design, Soil Types, and Manure Types*

This experiment was conducted as a laboratory incubation with four replications to determine the effect of different manure types on GHG emissions when applied as an organic amendment. Manure from cattle fed (1) a corn-based silage diet (CM), (2) a barley-based silage diet (BM), or (3) no manure (CK, control) were applied to three soil types: (1) Orthic Black Chernozem (OBC), (2) Dark Brown Chernozem (DBC), and (3) Orthic Gray Luvisol (OGL).

The two types of manure were produced from a feedlot study conducted at AAFC in Lethbridge, Alberta, Canada from an experiment by Chibisa and Beauchemin (2018). The 160 cattle were allocated to 16 pens with free access to water throughout the study. Manure from cattle on two of the treatments during the backgrounding phase was used in the study. One of the treatments was a diet consisting of 60% barley silage (barley silage diet) and the other was a diet consisting of 90% corn silage (corn silage diet). Both diets were formulated to supply approximately 13.5% crude protein. The cattle were fed their respective backgrounding diets for a period of 105 days, and then were transitioned for 28 days to a single low forage diet consisting of 8% barley silage, 5% supplement, and 87% dry rolled barley grain. The cattle received the finishing diet for 140 days. Another feed additive, Monensin (28 mg $kg^{-1}$), was included in all diets. The manure samples used for the incubation were freeze-dried to maintain chemical properties, consistency, and provide a more homogeneous mixture (Calderón et al., 2004).

Samples of the three soil types were collected from 0-15 cm in depth at sites in Alberta, Canada in late spring 2017. The soils were collected at Beaverlodge Research and Development Centre (54°58'51.1"N 117°24'30.7"W), Lacombe Research and Development Centre (52°26'49.9"N 113°45'23.2"W), and Agriculture and Agri-Food Canada’s Research and Development Centre (AAFC) in Lethbridge (49°42'22.4"N 112°45'29.2"W) for OGL, OBC, and

DBC soils, respectively. The OGL, OBC, and DBC soils had a clay loam texture, silty loam, and clay loam texture, respectively. The OGL and OBC soils were from fields under cereal-canola production for over 30 years and DBC soil was from a field under forage (alfalfa/grass) production. Soils were air-dried at room temperature in the lab and passed through a 2-mm sieve.

### *2.2.2 Incubation*

The samples were pre-incubated for seven days in the incubation chamber at 25 °C to allow settling from the disturbance created from setting up the experiment. Soil samples and soil samples with manure amendment were dried at 105 °C for 48 hours to determine the water content (Cai et al., 2016). Then 200 g (dry-weight basis) of soil were placed in each of 36 (three treatments x three soil types x four replications) 1-L Mason jars.

Each sample was adjusted to 60% water-filled pore space using a 20-mL syringe to distribute deionized water evenly over the surface of the soil inside the Mason jar. Water-filled pore space was calculated from the equation:

$$\%\ \text{water} - \text{filled pore space} = \frac{\text{SWC x BD}}{1-\left(\frac{\text{BD}}{\text{PD}}\right)} \tag{1}$$

where SWC is the soil water content (g $g^{-1}$), BD is bulk density (1.00 Mg $m^{-3}$), and PD is particle density (2.65 Mg $m^{-3}$) (Jones, 1983; Franzluebbers, 1999). The jars were covered with a piece of aluminum foil with four small holes to allow gas exchange and minimize water loss throughout the incubation.

Following the pre-incubation, 16.64 g of manure (dry-weight basis) was added to the top of the soil, not mixed with the soil. At the same time, deionized water was added to bring the soil and manure back to 60% water-filled pore space, and then the Mason jars were placed back into the chamber for the remainder of the 70-day incubation. The amount of manure added was comparable to the common field application of 16 Mg $ha^{-1}$ of organic manure, an amount typical for barley forage production (Sadeghpour et al., 2016).

Water lost by evaporation was replaced weekly to return samples to the prescribed water-filled pore space. The jars were adjusted to 60% water-filled pore space from day one to day 14, then adjusted to maintain 80% water-filled pore space from day 14 to day 70 to better understand both aerobic and anaerobic processes (Bremner and Shaw, 1958; Calderón et al., 2004).

### *2.2.3 Destructive Sampling - Physical and Chemical Analyses*

Destructive sampling collected the soil underneath the manure by scooping out the prescribed weight (see equation 1) with a spoon, then replacing the manure evenly across the surface. Total N, total C, organic carbon (OC), C/N ratio, microbial biomass C, and microbial biomass N were measured at the beginning, middle, and end of the incubation. Nitrate ($NO_3^-$-N), ammonium ($NH_4^+$-N), available N (sum of $NO_3^-$-N and $NH_4^+$-N) and GHG fluxes ($CO_2$, $N_2O$, and $CH_4$) were measured every 3 days for the first week, then on a weekly schedule for 2 weeks, and then on a bi-weekly basis for the remainder of the study.

Approximately 10 g (air-dried equivalent) of soil samples were collected on days 1, 4, 7, 14, 21, 35, 49, and 70 for determination of available N. An addition 15 g (air-dried equivalent) of soil samples were collected on days 1, 35, and 70 for determination of total N, C/N ratio, and microbial biomass. For measurements of microbial biomass and available N, soil samples were extracted with 0.5 M $K_2SO_4$ solutions at a 1:5 (w:v) soil-to-extract ratio and shaken at 250 rpm on a mechanical shaker for one hour. The solution was then filtered using Whatman No. 42 filter papers to gather the filtrate (Vance et al., 1987; Pattey et al., 2005). Microbial biomass was determined using a TOC-$V_{CSN}$ analyzer (Shimadzu, Kyoto, Japan) to analyze the filtrate, where differences between extractable C and N in fumigated and unfumigated samples representing microbial biomass C and microbial biomass N, respectively. Available N, specifically $NH_4^+$-N and $NO_3^-$-N, were measured by colorimetric analysis from the filtrate (Norman and Bremner, 1965).

Soil pH was determined using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) at a 1:5 soil to deionized water weight ratio (Cai et al., 2016). For soil with $pH > 7.2$, the inorganic C was removed by treating the soil with 6M HCl prior to determination of OC content (Ramnarine et al., 2011; Premrov et al., 2018). They were dried at 70 °C, ground (<2 mm) with a mortar and pestle, and analyzed for total C, and total N using dry combustion techniques in an automated CN analyzer (Carlo Erba, Milan, Italy) (Bremner, 1965).

### *2.2.4 Nondestructive Gas Sampling*

Gas samples were collected twice at each sampling day with a 20-mL syringe from each Mason jar and transferred to a pre-evacuated 12-mL exetainer vial at 0 hours. Then, after the Mason jar lid was sealed with a butyl rubber stopper, gas samples were collected again after 24 hours (Cai et al., 2016). The difference between these two measurements was used to calculate the fluxes (equation 2 below) throughout the incubation on day 1, 4, 7, 21, 35, 49, and 70. The carbon dioxide ($CO_2$), $CH_4$, and nitrous oxide ($N_2O$) emissions in the gas samples were measured with a Varian CP-3800 gas chromatograph (GC) (Varian Canada, Mississauga, Canada), equipped with a thermal conductivity detector (TCD), a flame ionization detector (FID), and an electron capture detector (ECD).

Atmospheric $CO_2$, $N_2O$, and $CH_4$ fluxes in mg C $kg^{-1}$ $h^{-1}$, μg N $kg^{-1}$ $h^{-1}$, and μg C $kg^{-1}$ $h^{-1}$ were calculated by comparing the gas density at the standard state to changes in gas concentrations during incubation. Flux was calculated from the equation (Van Zwieten et al., 2010):

$$\text{Flux} = \frac{\rho * \Delta c * V * 273K}{W * \Delta t * (273K + T)} \qquad (2)$$

where ρ = density at standard state, Δc = change in concentration during incubation (parts per billion by volume (ppbv)), Δt = incubation time (24 hours), W = soil mass (200 g), V = headspace volume in jar, and T = incubation temperature (25 °C).

Dissolved $N_2O$ was calculated according to Moraghan and Buresh (1977) and added to measured $N_2O$ flux to determine total atmospheric $N_2O$ flux. Total atmospheric $CO_2$ and $CH_4$ fluxes were represented by measured $CO_2$ and $CH_4$ fluxes only. Cumulative GHG emissions were calculated by summing gas fluxes over the incubation period. Cumulative anthropogenic GHG emissions were calculated as $CO_2$-equivalents as the sum of $N_2O$ and $CH_4$ emissions. Global warming potential coefficients of 265 and 28 were used for $N_2O$ and $CH_4$, respectively, to determine $CO_2$-equivalents (IPCC, 2014).

### *2.2.5 Statistical Analyses*

A two-sample t-Test was run to test for significant differences between the two different manure types and the initial soil properties. Before conducting the t-Test, the normality of distribution and homoscedasticity were checked with Shapiro and F-tests, respectively. All tests showed normal distribution and homoscedasticity, so no adjustments were made. A one-way ANOVA was used to test for significant differences between the three different soil types. Factorial multi-way ANOVAs were used to analyze the effect of soil type, organic manure, sampling time, and their interaction on cumulative GHG emissions and soil biogeochemical properties.

A two-way ANOVA was used to analyze the effect of soil type, manure type, and their interaction on cumulative GHG emissions. Before conducting the ANOVA, the normality of distribution and homogeneity of variance were checked with Shapiro and Bartlett tests, respectively. Data with non-homogeneous variance, such as $N_2O$, $CH_4$, and Anthropogenic GHG emissions were adjusted using AR(1) autoregressive models. The GHG emissions and initial soil type properties were analyzed using a Tukey-Kramer test. Relationships between initial soil properties and cumulative GHG emissions were examined using Spearman's rank correlation. Statistical significance was set at $\alpha = 0.05$ for all tests. All statistical analyses were performed using R software version 1.1.456 (R Foundation for Statistical Computing, Vienna, Austria).

## 2.3 Results & Discussion

### *2.3.1 $CO_2$ Emissions*

Non-amended soils had significantly lower cumulative $CO_2$ emissions than manure-amended soils (Fig. 2.5a). As labile C pools in the soil and manure were mixed, they were then mineralized by microbial activity (Kaiser et al., 2015). Because cattle manure increases microbial biomass, the OC contents of the amendments were much higher than those of the pure soils (Table 2.1) (Gregorich et al., 1998; Calderón et al., 2004; Pattey et al., 2005; Sampanpanish, 2012). This may provide insight into why the $CO_2$ emissions peaked at the beginning of the incubation and declined after day 7 (Fig. 2.4a).

Diet type had a significant impact on $CO_2$ emissions, with CM resulting in the significantly greatest flux for all soil types (Fig. 2.5a). There was a slightly greater C/N ratio in the CM (Table 2.1), potentially promoting mineralization of OC and that released greater $CO_2$ emissions (Calderón et al., 2004; van Vliet et al., 2007). While there was a statistically significant correlation between OC and $CO_2$ emissions, there was no significant difference in OC and C/N between CM and BM (Table 2.4), suggesting that another factor might be responsible for the difference.

The pH was slightly, yet significantly, higher in BM, signifying that the microbes release more $CO_2$ emissions potentially due to increased stress in acidic environments (Domsch and Anderson, 1993). The minimal difference in pH suggests other factors that were not measured, such as microbial species or carbon quality, could have been drastically altered by even small changes in pH. Depending on the microbial species, this could result in different effects to varying degrees (Ozbayram et al., 2018).

Soil type also showed a significant interaction with diet type for $CO_2$ emissions (Table 2.3). The differences in $CO_2$ emissions across soils types may be explained by the varying soil pH levels and OC levels. The study found a significantly positive correlation between $CO_2$ emissions and OC (Table 2.4). The OBC soil showed significantly greater $CO_2$ emissions than OGL and DBC soil (Fig. 2.5a), possibly from the statistically greater initial OC content (Table 2.1) that allowed for greater microbial activity (Pattey et al., 2005). Numerous researchers also found that pH levels are often positively correlated to higher microbial activity as the solubility of OC increases when the soil pH is raised by the addition of manure (Bremner and Shaw, 1958; Ajwa and Tabatabai, 1994; Curtin et al., 2016; Li et al., 2016a).

The $CO_2$ production rates were significantly greater in OGL and OBC soil types (Fig. 2.5a), both with pH <7 in comparison to the DBC soil type which had pH >7 (Table 2.1). Similar to Li et al. (2016a), there was a significantly positive correlation between $CO_2$ emissions and pH (Table 2.4). This may be because DBC had a pH closer to the pH of the manure types added, so the OBC and OGL soils also faced a greater increase in pH with the addition of the manure types

(Sadeghpour et al., 2016; Curtin et al., 2016; Li et al., 2016a). The greater change in pH could have led to increased solubilization of OC previously stabilized under more acidic conditions, allowing for enhanced microbial activity and $CO_2$ efflux (Curtin et al., 2016).

### *2.3.2 $N_2O$ Emissions*

Upon application of both manure types to the soil, there was a sharp peak of $N_2O$ emissions (Fig. 2.4b). High N input from manure addition may have formed $NH_4^+$-N from microbial mineralization interactions (Bremner, 1997; Dalal et al., 2003; Thangarajan et al., 2013), as there was a strong correlation between $N_2O$ emissions and $NH_4^+$-N (Table 2.4) This correlation between $N_2O$ emissions and $NH_4^+$-N was also observed by Li et al. (2016a). They concluded that initial nitrification likely occurred, as the project began under aerobic conditions, and with high $NH_4^+$-N and no $NO_3^-$-N in both BM and CM amendments (Table 2.1) (Li et al., 2016a). The initial nitrification reaction is predicted to have ended by day 21 of our experiment, where little $NH_4^+$-N is seen throughout the rest of the incubation (Fig. 2.4b). Once there was sufficient $NO_3^-$-N from said nitrification, denitrification may have simultaneously occurred; however, this is less certain, as the correlations only observed the soil properties on day one, where little $NO_3^-$-N was present (Čuhel et al., 2010).

Similar to the Li et al. (2016a) incubation, another small jump in $N_2O$ emissions occurred around day 21 (Fig. 2.4b) with a corresponding increase in $NO_3^-$-N (Fig. 2.2b). However, this was followed by another increase in $N_2O$ emissions around day 49 (Fig. 2.4b) with another corresponding $NO_3^-$-N peak around day 35 (Fig. 2.2b). This second peak may have occurred because the remainder of incubation after day 14 occurred under purely anaerobic (80% water-filled pore space) conditions. The remaining $N_2O$ emissions produced are likely to be a result of denitrification once the initial lag period ended due to reduced soil redox potential (Franzluebbers, 1999; Sampanpanish, 2012; Thangarajan et al., 2013). Without anaerobic conditions present, the addition of manure to the soil may have only resulted in $N_2O$ emissions for the first few weeks (Bhandral et al., 2007; Li et al., 2016a).

Diet type had a significant impact on $N_2O$ emissions, as the BM-amended soils had significantly greater $N_2O$ emissions than CM soils in the DBC soil (Fig. 2.5b) The differences may be explained by the higher $NH_4^+$-N (and thus, available N, as there is no $NO_3^-$-N present) in the BM (Table 2.1), which is significantly correlated to $N_2O$ emissions through nitrification (Čuhel et al., 2010). However, there were no statistically significant differences in $NH_4^+$-N between manure from cattle fed the two diets (Table 2.4).

Nevertheless, the relationship between pH and $NH_4^+$-N could help explain the relationship between $N_2O$ emissions and $NH_4^+$-N (Čuhel et al., 2010; Van Zwieten et al., 2010). The pH of soils has a direct effect on ammonia ($NH_3$) volatilization in soils which increases $NH_4^+$-N (du Plessis and Kroontje, 1964; Bremner, 1997), thus the small, but significantly, higher pH of the BM amendment may explain the higher $NH_4^+$-N (Table 2.1). Another potential source of $N_2O$ emissions is chemodenitrification, the decomposition of nitrite in acidic soils by volatilization of nitrite ($NO_2^-$-N) and fixation of $NO_3^-$-N by SOM, that could account for the greater $N_2O$ emissions based on soil pH differences (Bremner, 1997).

In addition to pH differences, the difference in the $NH_4^+$-N may also have resulted from differences in the form of N (fecal vs. urinary) excreted from animals fed the respective diets. Although the corn silage was lower in crude protein than the barley silage (9.1 vs. 10.7% of dry matter), both diets were formulated to be iso-nitrogenous (approximately 13.5% crude protein, dry matter basis) (Chibisa and Beauchemin, 2018). Urea and canola meal were used to contribute the additional protein for the corn diet, and thus the concentration of soluble protein and amino acids provided to rumen microbes differed between diets (Chibisa and Beauchemin, 2018). These differences in diet composition may have affected the proportion of N in feces versus urine, with N in urine present in a more volatile form than N in feces (Ørskov, 1986). However, digestibility and excretion of N were not measured in the animal study.

Soil type also showed a significant interaction with soil type, as the OBC soils had significantly higher OC than DBC or OGL soils (Table 2.1). This suggests that additional denitrification may have contributed to $N_2O$ emissions as well as nitrification, as nitrification will mostly occur in soils low in OC (Bremner and Shaw, 1958). This is because, at higher OC, microbes switch from using $NH_4^+$-N as their primary source to using $NO_3^-$-N, preventing the nitrification conversion and switching to denitrification (Calderón et al., 2004; Dijkstra et al., 2006; Cai et al., 2016). Because the majority of the incubation was set under anaerobic conditions, denitrification was likely the predominant process. For this reason, OBC had higher OC and likely predominantly performed denitrification - potentially explaining why OBC had significantly higher $N_2O$ emissions than OGL or DBC soils (Fig. 2.5b) (Čuhel et al., 2010; Van Zwieten et al., 2010).

There was a significant interaction between soil and manure type (Table 2.3). The BM resulted in the greatest $N_2O$ emissions for DBC and OGL soil types, although the difference was not significant for OGL soils (Fig. 2.5b). Weier et al. (1993) and Van Zwieten et al. (2010) found that soil texture plays a role in determining which N process would occur. Different soil texture compositions required different water-filled pore space levels to be considered anaerobic because smaller pore size leads to greater water retention (Weier et al., 1993). The OGL and DBC are both clay loam soils, in comparison to the silty clay OBC. The greater clay percentage and smaller pore spaces could have contributed to the greater $N_2O$ emissions from denitrification in the OBC soil. This difference in texture could explain why OBC soils had a higher (but not significant) flux from the CM amendment in comparison to OGL and DBC soils.

However, caution must be exercised when applying this knowledge to field studies as the soil was air-dried prior to the incubation. Water stress on soil microbiota has been found been to be greater in coarse-textured soils in comparison to fine-textured soils (Kaiser et al., 2015). The reason clayey soils are less susceptible to water stress is that the fine-textured soils store more OM, and thus contain a greater microbial population (Kaiser et al., 2015). There was a significant correlation between microbial biomass and $N_2O$ emissions (Table 2.4). Therefore, the OBC soil (sandy loam) that does not face water stress may have even greater $N_2O$ emissions than reported in this study.

### *2.3.3 $CH_4$ Emissions*

The greatest $CH_4$ emissions came from manure-amended soils, and very little $CH_4$ emissions from CK amendments across all soil types (Fig. 2.5c) Aerobic soils act as sinks for $CH_4$ production if soils are well aerated, so $CH_4$ emissions are often negligible (Thangarajan et al., 2013). Similar to $CO_2$ and $N_2O$ emissions, $CH_4$ emissions are produced by organic decomposition resulting from microbial activity; however, $CH_4$ is only produced in anaerobic conditions (Eckard et al., 2010; Sampanpanish, 2012; Thangarajan et al., 2013). All $CH_4$ emissions were positive, except towards the end of the incubation around day 60 (Fig. 2.4c).

The reason for the positive $CH_4$ emission as described by Pattey et al. (2005) and Sampanpanish (2012) is that the addition of organic matter may seal the surface of the soil, reducing the pore space and corresponding available $O_2$ for the microorganisms. The reason for the decline in flux towards the end of the experiment may be a decomposition of organic matter over time. If the manure becomes more porous from decomposition, methanogenesis would decline (Pattey et al., 2005). Pattey et al. (2005) did not report a decline in $CH_4$ emission until approximately day 220 of their experiment. Because of fluctuations of $CH_4$ emissions throughout the entire experiment (Fig. 2.4c), it is possible the experiment did not complete decomposition equilibrium.

Diet type had a significant impact as the BM-amended OGL and OBC soils, with the exception of DBC soil, had significantly higher $CH_4$ emissions than CM amendments (Fig. 2.5c). The CM had a significantly lower pH than BM (Table 2.1), which was also significantly positively correlated to $CH_4$ emissions (Table 2.4). The differences in pH to may relate to the site of digestion of starch within the digestive tract of the animals (Spicer et al., 1986; Ørskov, 1986). The corn silage diet contained less starch (27 vs. 35% of dry matter) than the barley diet, but starch from corn is less digestible in the rumen, resulting in greater hind-gut fermentation (Gibb and McAllister, 2003; Chibisa and Beauchemin, 2018). Greater post-ruminal digestion lowers fecal pH (Ørskov, 1986).

The $CH_4$ emissions showed significant differences in manure type, soil type, and their interaction (Table 2.3). However, unlike the other two soils which had statistically higher $CH_4$ emissions for BM-amendments, the DBC soil showed slightly higher (but not significant) $CH_4$ emissions from CM amendment (Fig. 2.5c). In fact, there was no statistical difference between the amended and unamended soil for the DBC soil type. The DBC soil had a higher pH than the other soils (Table 2.1), suggesting the potential to promote methanogenic bacteria activity (Sampanpanish, 2012). Neutral soil has been shown to improve the growth of methanogens (Bremner and Shaw, 1958; Thangarajan et al., 2013). Boadi et al. (2004) also indicate that there are specialized methanogens that are isolated in the rumen that may play a role in only certain soil pH ranges. Perhaps the methanogens only interacted with soils with a neutral pH as observed in the DBC soils, lowering the effect of the amendments. More research using 16S rRNA analysis is required to better understand these microorganisms (Boadi et al., 2004; Nikkhah, 2012).

## 2.4 Conclusions

Modifying the diet composition for beef cattle changed the corresponding manure properties, and thus, the release of GHGs from soils amended with the manure. Overall, soils amended with CK had the lowest $CO_2$, $N_2O$, and $CH_4$ emissions. Soils amended with CM had the highest $CO_2$ emissions while those amended with BM had the highest $N_2O$ emissions. Methane emissions were highest for BM-amended soils in OBC and OGL soil types. Manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected GHG emissions. The interaction of soil and manure may come from the pH of soils, which has a direct effect on $NH_3$ volatilization, increasing $NH_4^+$-N. However, because the pH difference was minimal, it is hypothesized that these results may be a result of something not investigated in this study. The interaction of soil and manure lowered the $N_2O$ and $CH_4$ emissions, meaning that in terms of equal amounts of manure produced, cattle fed corn- rather than barley-based diet may help reduce the total GHG emissions from manure, specifically when amended to coarse-textured soils.

As the amount of manure excreted is not considered, the net GHG from these feeding regimes cannot be determined. Moreover, the results from this laboratory incubation are limited in scope because rewetting air-dried soils under field conditions may change microbial functions. Thus, future work should investigate the effect of additional soil types on GHG emissions in the field following manure application from cattle fed corn- or barley-based diet. Additional emissions management of manure such as stockpiling, transporting of manure, etc. should also be considered.

## 2.5 Tables and Figures

**Table 2.1.** Chemical properties of soil and manure types used in incubation (means ±SE) (*n* = 3).

| Parameter | pH* | TN ($g\ kg^{-1}$) | OC ($g\ kg^{-1}$) | C/N | $NH_4^+$-N ($mg\ kg^{-1}$) | $NO_3^-$-N ($mg\ kg^{-1}$) | AN ($mg\ kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Manure Type | | | | | | | |
| CM | $8.31 \pm 0.02^a$ | $17.0 \pm 2.39^a$ | $238 \pm 33.4^a$ | $14.1 \pm 0.61^a$ | $51.6 \pm 0.53^a$ | UDL** | $51.6 \pm 0.53^a$ |
| BM | $8.42 \pm 0.01^b$ | $18.2 \pm 0.20^a$ | $247 \pm 14.9^a$ | $13.6 \pm 0.70^a$ | $54.9 \pm 0.71^a$ | UDL** | $54.9 \pm 0.71^a$ |
| Soil Type | | | | | | | |
| DBC | $7.26 \pm 0.03^a$ | $1.51 \pm 0.06^c$ | $18.0 \pm 0.58^c$ | $15.9 \pm 0.28^a$ | $1.42 \pm 0.15^b$ | $8.43 \pm 0.50^a$ | $9.85 \pm 0.54^b$ |
| OBC | $5.75 \pm 0.05^b$ | $5.05 \pm 0.22^a$ | $61.6 \pm 2.94^b$ | $12.2 \pm 0.06^c$ | $11.0 \pm 0.99^a$ | $3.93 \pm 0.43^b$ | $16.2 \pm 0.59^a$ |
| OGL | $4.92 \pm 0.02^c$ | $2.29 \pm 0.08^b$ | $32.9 \pm 1.35^a$ | $14.4 \pm 0.30^b$ | $1.08 \pm 0.10^b$ | $7.63 \pm 0.34^a$ | $8.48 \pm 0.34^b$ |

**Note**: Abbreviations: AN, available nitrogen; TN, total nitrogen; OC, organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. Soil type: DBC, Dark Brown Chernozem; OBC, Orthic Black Chernozem; OGL, Orthic Gray Luvisol. Manure type: BM, manure from cattle fed a typical backgrounding diet; CM, manure from cattle fed a corn-based backgrounding diet. Letters denote significant differences between treatments, treatments that do not share the same letter are significantly different from each other. *Soil pH was measured in 1:5 soil:water ratio. **Under detection limit

**Table 2.2.** Results of multi-way ANOVAs (p-values) testing the effects of soil type (S), manure type (M), time (T) and their interactions on soil biogeochemical properties (*n* = 4).

| Source of Variation | S | M | T | S x M | S x T | M x T | S x M x T |
|---|---|---|---|---|---|---|---|
| $NH_4^+$-N | < 0.001 | < 0.001 | 0.018 | < 0.001 | < 0.001 | < 0.001 | 0.012 |
| $NO_3^-$-N | < 0.001 | < 0.001 | 0.060 | < 0.001 | < 0.001 | 0.015 | 0.257 |
| AN | < 0.001 | < 0.001 | 0.780 | < 0.001 | < 0.001 | 0.025 | 0.113 |
| TN | < 0.001 | < 0.001 | 0.626 | < 0.001 | 0.937 | 0.067 | 0.121 |
| C/N Ratio | < 0.001 | < 0.001 | 0.330 | < 0.001 | < 0.001 | < 0.001 | 0.098 |
| OC | < 0.001 | < 0.001 | 0.185 | < 0.001 | 0.531 | < 0.001 | < 0.001 |
| MBC | < 0.001 | < 0.001 | 0.933 | < 0.001 | < 0.001 | 0.600 | < 0.001 |
| MBN | < 0.001 | < 0.001 | 0.704 | < 0.001 | 0.044 | < 0.001 | 0.147 |

Abbreviations: AN, available nitrogen; TN, total nitrogen; OC, organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

**Table 2.3.** Results of two-way ANOVAs (p-values) testing the effects of manure type (M), soil type (S), and their interactions (S x M) on cumulative GHG emissions (*n* = 4).

| Source of Variation | S | M | S x M |
|---|---|---|---|
| $CO_2$ Emissions | < 0.001 | < 0.001 | < 0.001 |
| $N_2O$ Emissions | < 0.001 | < 0.001 | < 0.001 |
| $CH_4$ Emissions | 0.155 | < 0.001 | < 0.001 |
| Anthropogenic GHG Emissions | 0.047 | < 0.001 | < 0.001 |

**Table 2.4.** Pearson's correlation coefficients of cumulative $CO_2$, $N_2O$, and $CH_4$ emissions to initial soil properties (*n* = 12).

| Variable | pH | OC | TN | C/N | $NH_4^+$-N | $NO_3^-$-N | AN | MBC | MBN |
|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ | 0.81* | 0.57** | 0.62** | -0.10 | 0.50* | -0.34 | 0.41 | 0.25 | 0.15 |
| $N_2O$ | 0.31 | 0.49* | 0.61** | -0.20 | 0.72** | 0.05 | 0.76** | 0.59** | 0.77** |
| $CH_4$ | 0.52* | 0.05 | 0.12 | -0.11 | 0.73** | -0.24 | 0.61** | 0.15 | 0.47* |

Abbreviations: AN, available nitrogen; TN, total nitrogen; OC, organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. *, $P < 0.05$; **, $P < 0.01$.

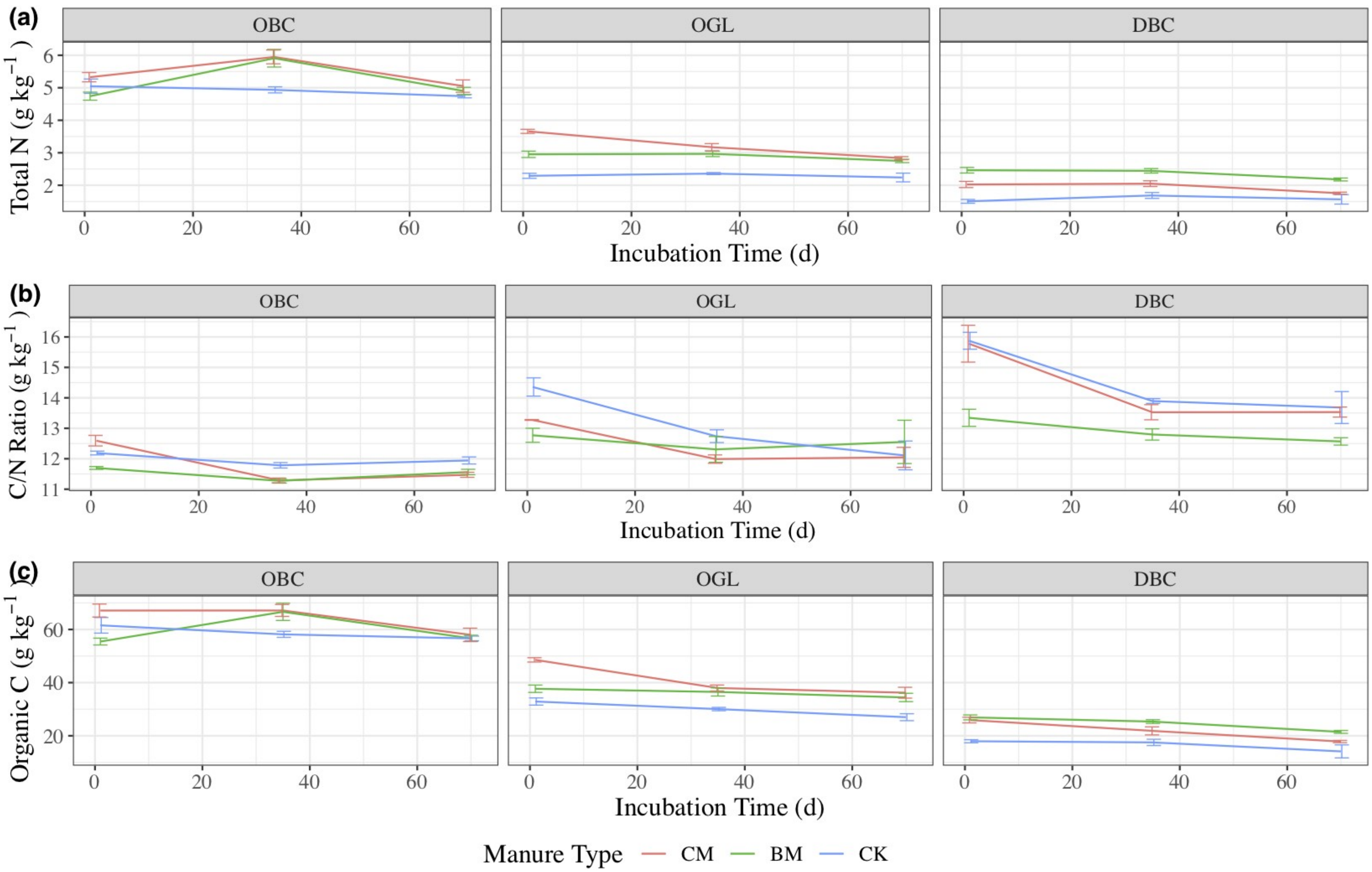

**Fig. 2.1** Effects of manure type on (a) total nitrogen, (b) carbon/nitrogen ratio, and (c) organic carbon in various soil types throughout the incubation. Soil type: DBC, Dark Brown Chernozem; OBC, Orthic Black Chernozem; OGL, Orthic Gray Luvisol. Manure type: BM, manure from cattle fed a typical backgrounding diet; CM, manure from cattle fed a corn-based backgrounding diet; CK, non-amended soil. Vertical bars indicate standard errors of the means (*n* = 4).

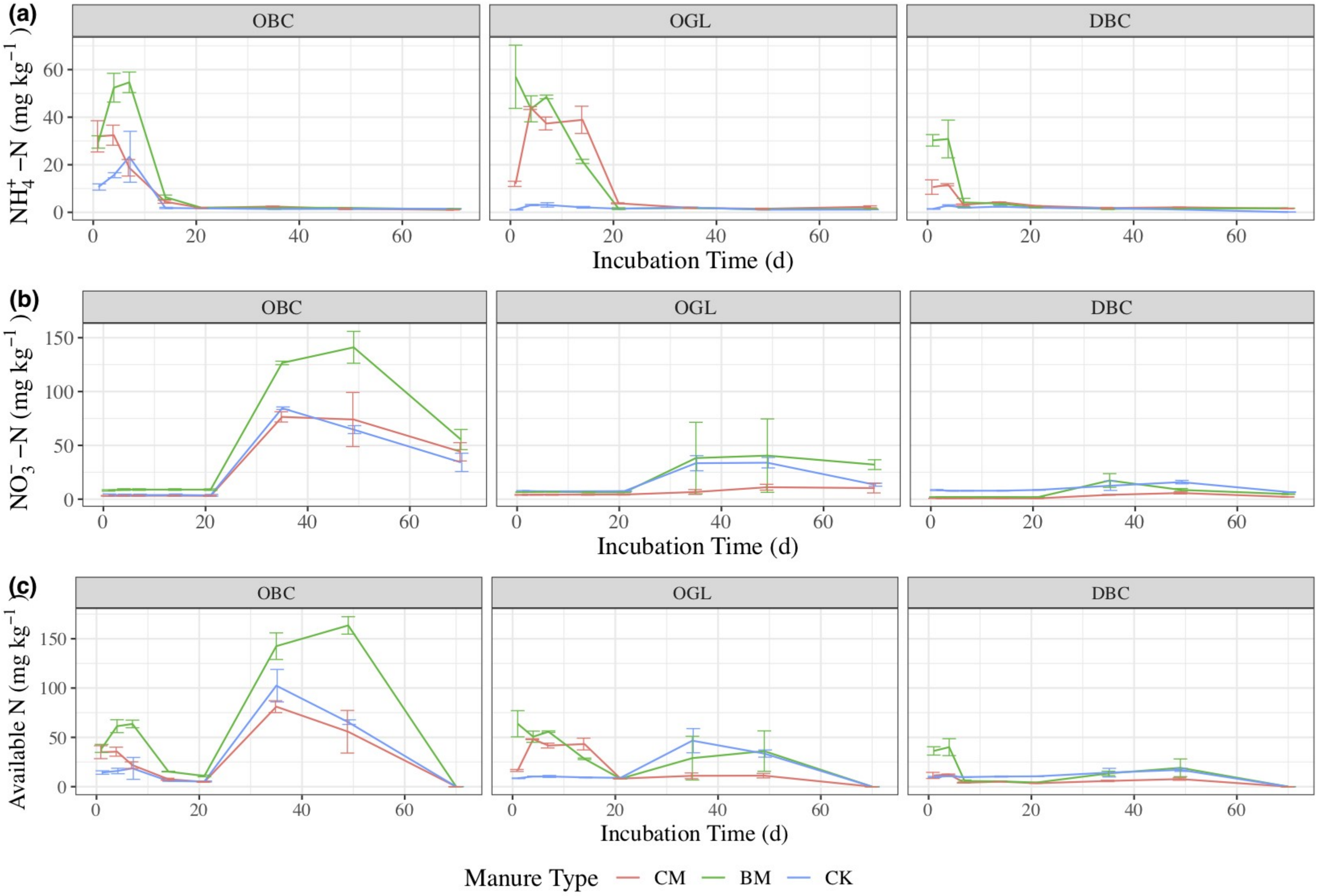

**Fig. 2.2** Effects of manure type on (a) ammonium, (b) nitrate, and (c) available nitrogen in various soil types throughout the incubation. Soil type and manure type codes are identical to Fig 2.1. Vertical bars indicate standard errors of the means (*n* = 4).

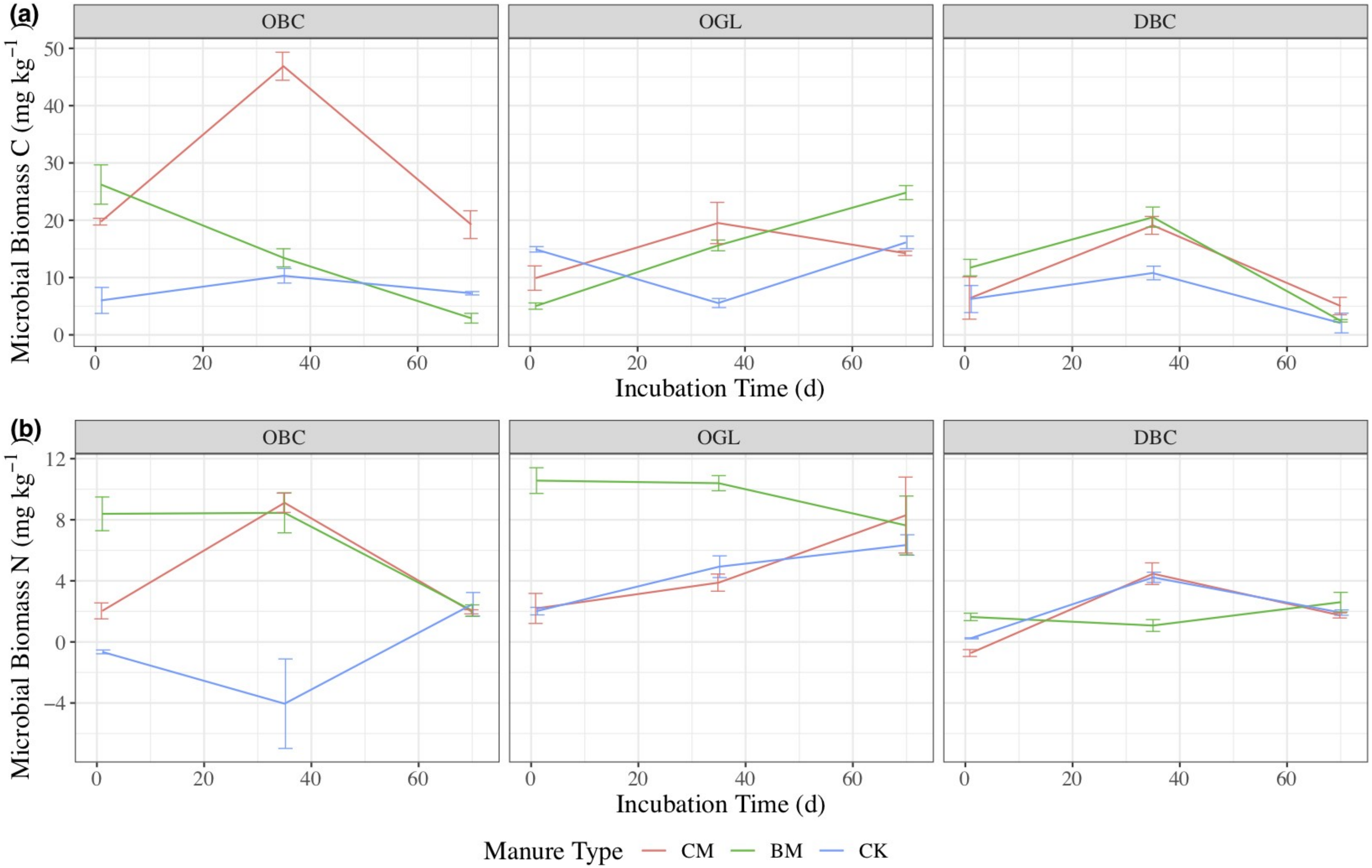

**Fig. 2.3** Effects of manure type on (a) microbial biomass C and (b) microbial biomass N in various soil types throughout the incubation. Soil type and manure type codes are identical to Fig 2.1. Vertical bars indicate standard errors of the means (*n* = 4).

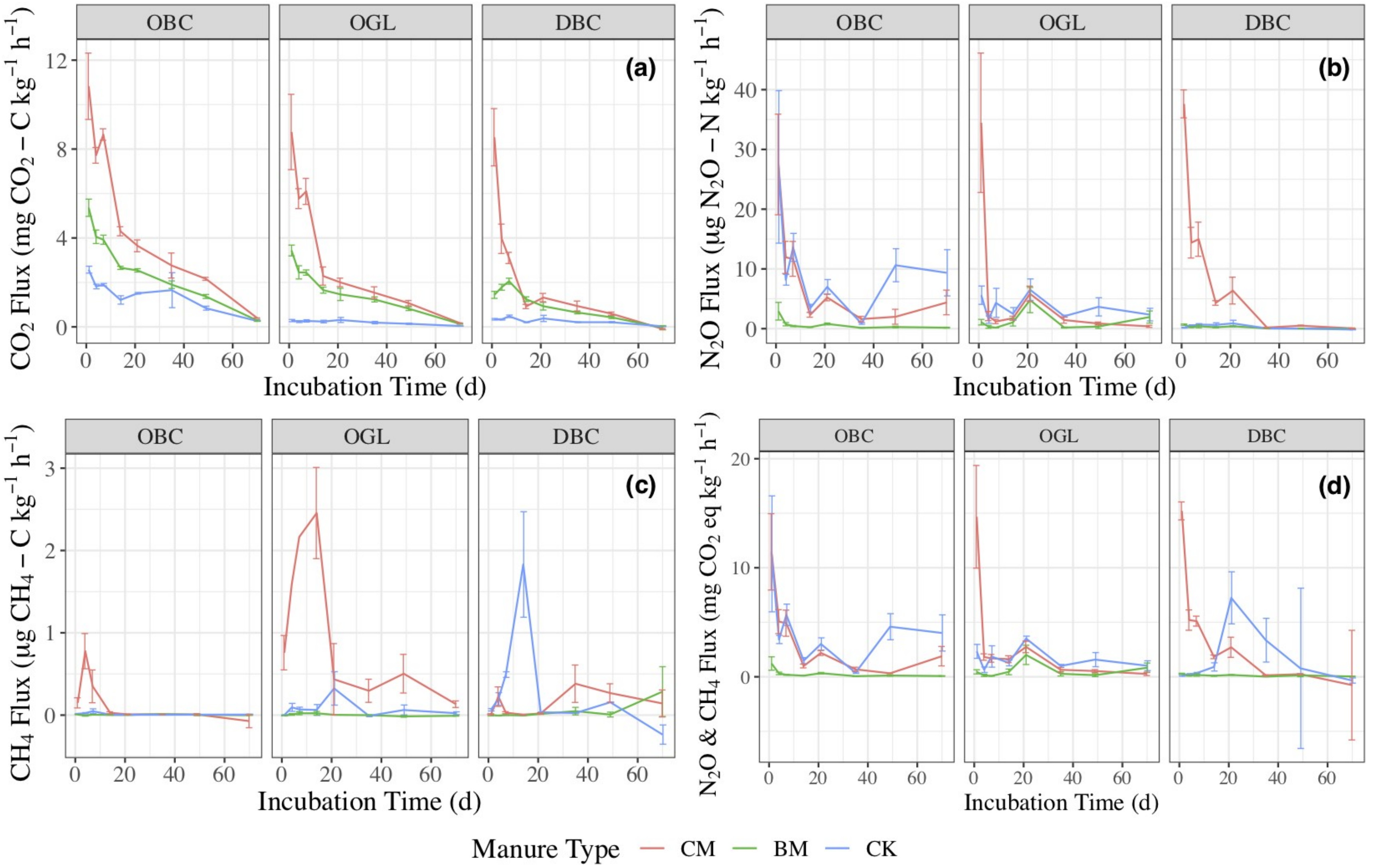

**Fig. 2.4** Effects of manure type on (a) $CO_2$, (b) $N_2O$, (c) $CH_4$, and (d) anthropogenic GHG emissions in various soil types throughout the incubation. Soil type and manure type codes are identical to Fig 2.1. Vertical bars indicate standard errors of the means ($n = 4$).

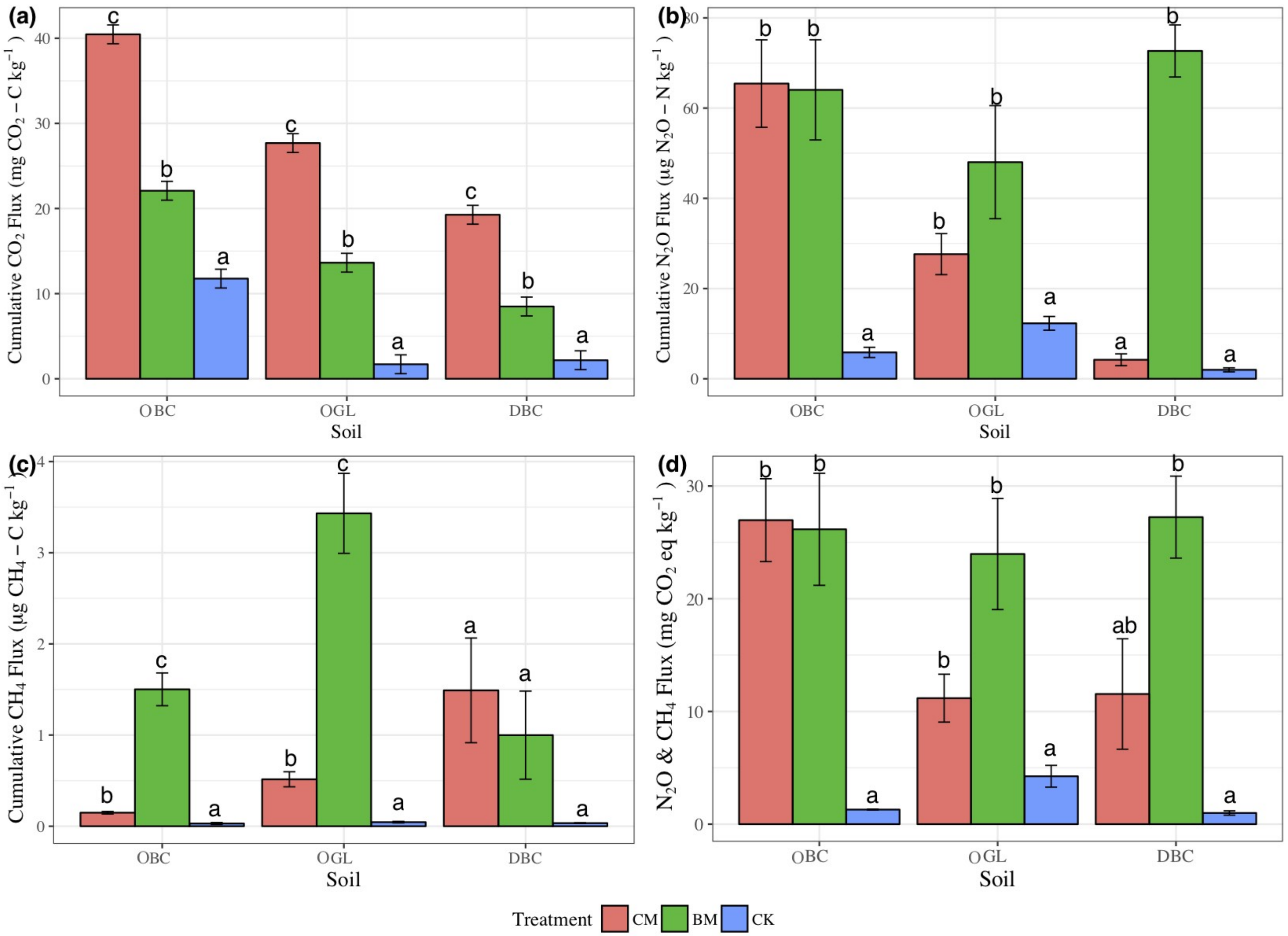

**Fig. 2.5** Effects of manure type on cumulative (a) $CO_2$, (b) $N_2O$, (c) $CH_4$, and (d) anthropogenic GHG emissions in various soil types. Soil type and manure type codes are identical to Fig 2.1. Letters denote significant differences between treatments, treatments that do not share the same letter are significantly different from each other. Vertical bars indicate standard errors of the means (*n* = 4).

# Chapter 3. Composted manure from cattle fed with 3-nitrooxypropanol affected greenhouse gas emissions from three soil types in an incubation experiment

## 3.1 Introduction

Emission of greenhouse gases (GHG) is of growing importance and cattle production produces more GHG than any other livestock. While the massive amount of manure from farms poses many environmental problems, using cattle manure as an organic amendment is important to improving soil fertility (Hütsch et al., 1993b; de Vries and de Boer, 2010; Reeve et al., 2012; Li et al., 2016a). Cattle manure amendment increases soil aggregation, water holding capacity, and microbial activities (Reeve et al., 2012). New research is focusing on feeding management practices such as the use of feed additives and changing the composition of livestock feed to alter the properties of manure to reduce consequent GHG emission when the manure is applied to the soil (Haisan et al., 2014; Lopes et al., 2016).

Methane ($CH_4$) has 28 times greater greenhouse effect than carbon dioxide ($CO_2$), so reducing $CH_4$ production is essential for environmental sustainability (Smith et al., 2003; IPCC, 2014). The 3-nitrooxypropanol (3NOP) is a cattle feed additive created to help mitigate $CH_4$ emission from enteric fermentation as part of ruminant animals' normal digestive process (Reynolds et al., 2014; Vyas et al., 2018). The 3NOP inhibits the activity of methyl coenzyme-M reductase, the enzyme that forms $CH_4$, by using a reducible nitrate ($NO_3^-$-N) group to oxidize nickel (Ni) (Reynolds et al., 2014; Lopes et al., 2016; Jayasundara et al., 2016; Datta et al., 2017). Use of 3NOP is not reported to reduce the number of bacteria, protozoa, and methanogens, but rather to alter the function of specific microorganisms (Romero-Perez et al., 2014).

The 3NOP is one of the most successful inhibitors for enteric methane; Haisan et al. (2014) and Jayasundara (2016) found it to decrease emissions up to 28-33%. Moreover, 3NOP has the potential to allow for increased milk production as it reduces 2-12% of potential energy loss from $CH_4$ production (Haisan et al., 2014; Romero-Perez et al., 2015; Lopes et al., 2016). However, it is not clear how the addition of 3NOP will affect the resulting GHG emissions when the cattle manure is applied as an organic amendment to various soil types.

Another way to reduce GHG emissions is by altering livestock manure management practices. One such example is composting, designed to transformed biological waste into a stable humus-like material (Larney and Hao, 2007; Jayasundara et al., 2016). Only using local manure would mean excessive application and create environmental issues, therefore manure is often shipped great distances. Composting manure helps reduce the noxious smells, costs, and volumes of manure needed to be transported (Schlegel, 1992; Ros et al., 2006; Larney and Hao, 2007; Sadeghpour et al., 2016). Fresh manure consists of more than 80% water, resulting in large quantities of manure required for fertilization (Schlegel, 1992; Weil and Brady, 2017). Because of its liquid consistency, contamination through nitrate ($NO_3^-$-N) leaching is also common (Schlegel, 1992; Hadas and Portnoy, 1994).

Composting oxidizes ammonium ($NH_4^+$-N) to $NO_3^-$-N through nitrification (Yamamoto et al., 2010). Composting converts easily degradable C and N to GHG; the resulting manure has increased $NO_3^-$-N and decreased available N, pH, organic matter, $NH_4^+$-N, and C/N ratio (Schlegel, 1992; Wang et al., 2012; Eldor, 2015; Li et al., 2016a; Weil and Brady, 2017). As composting stabilizes the organic matter, the degradation rate decreases over the composting process as easily degradable materials are converted to $CO_2$ (Larney and Hao, 2007).

Composting can even help reduce pathogens, parasites, fly breeding, and weed seeds often found in manure (Hadas and Portnoy, 1994; Hao et al., 2001; Larney and Hao, 2007). However, some composted manure from cattle fed feed additives has a greater risk for N and P losses from the additional N in the supplement (Li et al., 2016b). Hao et al. (2001) discovered that greater aeration allows for changes in C and N dynamics that can alter GHG emissions. Manure from different feed, such as cows fed wheat dried distillers' grains with solubles, applied to soil is reported to have significantly lower GHG emissions when the amendment is in compost form compared to stockpiled form (Li et al. (2016a); however, the application of manure and composted manure to the soil from cattle fed with 3NOP additive has yet to be investigated.

There is a need to better define the interaction of manure from cattle fed 3NOP added to various soil types as organic amendments to improve sustainable management operations (Vyas et al., 2018). Therefore, the objectives of this experiment were to (i) determine the effect of the different treatments (composting, stockpiling, and 3NOP additive) on soil properties and GHG emissions and (ii) determine any trends among C and N dynamics and GHG emissions in the soil and management strategies during an 84-day incubation experiment. By clearly defining the impact of 3NOP additive in cattle diet on GHG emissions and soil biogeochemistry after manure /compost is applied to soil, these results will help to develop and recommend best manure management practices that enhance future beef industry feeding practices.

## 3.2 Materials and Methods

### *3.2.1 Experimental Design and Treatments*

The manure and soil type effects on GHG emissions in manure-amended soils were investigated using a completely randomized design (CRD). This is based on a 4 x 3 factorial experiment, with four manure treatments: soil amended with (1) stockpiled manure from cattle on traditional western feedlot diet (BM), (2) stockpiled manure from cattle on traditional western feedlot diet with 3NOP additive (3NOPM), (3) compost produced using manure in treatment (2) (3NOPC), and (4) a non-amended control (soil without manure) (CK); and three soil types: (1) Dark Brown Chernozem (DBC), (2) Orthic Black Chernozem (OBC), and (3) Orthic Gray Luvisol (OGL). Those treatment combinations were replicated four times.

The different manure types came from a feedlot study conducted by Vyas et al. (2018) at Agriculture and Agri-Food Canada (AAFC). Two hundred and forty crossbred yearling steers were used in a 238-day feeding trial. They were fed high-forage diets based on barley silage for the first 105 days then transitioned for 28 days to a high grain finishing diet for the remaining 105 days. One of the treatments involved the traditional western cattle diet and another the same diet supplemented with 200 mg $kg^{-1}$ dry matter 3NOP for backgrounding and 125 mg $kg^{-1}$ DM for the finishing phase. The 3NOP was homogeneously mixed into the total mixed ration daily. The 3NOPM was then composted (3NOPC) in open compost windrows. All manure samples used for this incubation were collected from the finishing stage and were sent to the University of Alberta fresh for incubation and analysis.

Surface soil samples (0-15 cm) used in this incubation experiment include: (1) an Orthic Dark Brown Chernozem (DBC) from AAFC in Lethbridge, AB, (49°42'22.4"N 112°45'29.2"W) with a clay loam texture; (2) an Orthic Black Chernozem (OBC) from Virden, MB, with a sandy loam texture; and (3) an Orthic Gray Luvisol (OGL) from Beaverlodge, AB (54°58'51.1"N 117°24'30.7"W) with a clay loam texture. The soils were collected from an alfalfa/grass pasture, prairie, and cereal-canola production for DBC, OBC, and OGL soil types respectively. After collection, the soil was air-dried at room temperature, passed through a 2-mm sieve to remove debris, mixed for homogeneity, and stored at room temperature (Kaiser et al., 2015). Properties of the amendments and soils used in the study are presented in Table 2.1.

### *3.2.2 Soil Incubation*

Soil samples were dried at 105 °C for 48 hours to determine the soil water content. Water-filled pore space (WFPS) was then calculated from:

$$\%\ \mathrm{WFPS}\ = \frac{\mathrm{SWC\ x\ BD}}{1-\left(\frac{\mathrm{BD}}{\mathrm{PD}}\right)} \qquad (1)$$

where SWC is the soil water content (g $H_2O$ $g^{-1}$ dry soil), BD is bulk density (Mg $m^{-3}$), and PD is particle density (2.65 Mg $m^{-3}$) (Franzluebbers, 1999; Eldor, 2015).

The incubation took place in a Forma Diurnal Growth Chamber-Model 3740 (Thermo Fisher Scientific, 112 Colonnade Road Nepean, ON K2E 7L6, Canada) at 25 °C. Two hundred grams (dry-weight basis) of soil was weighed and placed in one of the 48 (four treatments x three soil types x four replications) 1-L Mason jar. Water-filled pore space was adjusted to 60% using a 20 mL syringe to distribute deionized water evenly over the surface of the soil, then a piece of aluminum foil with four small pinholes was placed on top of the jar to allow for gas exchange, but to minimize water loss. The samples were pre-incubated in the incubation chamber for 7 days.

Immediately after pre-incubation, 16.64 g of fresh manure (dry-weight basis) was individually mixed into the soil. This amount is comparable to the common field application of 16.1 Mg $ha^{-1}$ of the organic amendment (typical for barley forage production) (Li et al., 2016a). At the same time, the water-filled pore space was adjusted to 60% for the first two weeks using a 20-mL syringe, then adjusted to 80% water-filled pore space for the remainder of the project.

The water-filled pore space was increased to better understand aerobic and anaerobic microbial processes that give rise to various GHG emission processes (Gregorich et al., 2005).

### 3.2.3 *Nondestructive Gas Sampling*

The GHG fluxes (carbon dioxide ($CO_2$), nitrous oxide ($N_2O$), and $CH_4$) were measured every 3 days for the first week, then on a weekly schedule for 2 weeks, then biweekly for the remainder of the study (day 1, 4, 7, 14, 21, 35, 38, 42, 49, 63, and 84). During non-disruptive sampling, gas samples were collected twice by first sealing each Mason jar with a lid containing a butyl rubber stopper. With a 20-mL syringe, air samples were collected at time 0 and transferred to a pre-evacuated 12 mL exetainer. After 24 hours, another set of gas samples were collected in the same manner and the butyl rubber stoppers were replaced with aluminum foil with four holes to allow gas exchange. A Varian CP-3800 gas chromatograph (GC) (Varian Canada, Mississauga, Canada), equipped with a thermal conductivity detector (TCD), a flame ionization detector (FID), and an electron capture detector (ECD) measured the $CO_2$, $CH_4$, and $N_2O$ concentrations, respectively, in the gas samples.

The carbon dioxide ($CO_2$), nitrous oxide ($N_2O$), and methane ($CH_4$) fluxes, in units of mg $CO_2$-C $kg^{-1}$ $h^{-1}$, μg $N_2O$-N $kg^{-1}$ $h^{-1}$, and μg $CH_4$-C $kg^{-1}$ $h^{-1}$ respectively, were calculated by comparing the gas density at standard state to change in gas concentrations (Li et al., 2016a). Flux was calculated from the equation:

$$F = \frac{\rho * \Delta c * V * 273}{W * \Delta t * (273 + T)} \tag{2}$$

where ρ = density at standard state, Δc = change in concentration during incubation (ppbv), Δt = incubation time (24 hours), W = soil mass (200 g), V = headspace volume in Mason jar, and T = incubation temperature (25 °C). Dissolved $N_2O$ was calculated according to Moraghan and

Buresh (1977) and added to measured $N_2O$ flux to determine total atmospheric $N_2O$ flux. Total atmospheric $CO_2$ and $CH_4$ fluxes were determined by measured $CO_2$ and $CH_4$ fluxes only. Cumulative GHG emissions were calculated by summing gas fluxes over the 84-day incubation period. The anthropogenic $CO_2$-eq was calculated using the GWP coefficients of 265 and 28 for $N_2O$ and $CH_4$ respectively for the 100-year time frame used in the calculation (IPCC, 2014).

### *3.2.4 Destructive Sampling - Physical and Chemical Analyses*

Soil total N, total C, organic C (OC), and C/N ratio were measured at the beginning, middle, and end of the incubation, while available N ($NO_3^-$-N and $NH_4^+$-N) was measured every 3 days for the first week, then on a weekly schedule for 2 weeks, then biweekly for the remainder of the study. Soil pH was measured after the sample was shaken for 30 min in a 1:5 soil weight to deionized water volume ratio on a reciprocal shaker and allowed to settle for 30 min. A pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) was used to determine the pH. For soil with pH > 7.2, the inorganic C was removed by treating the soil with 6M HCl prior to determination of SOC content (Ramnarine et al., 2011; Premrov et al., 2018).

Approximately 10 g of soil samples were collected every 3 days for the first week, then on a weekly schedule for 2 weeks, then biweekly for the remainder of the study and approximately 20 g of soil samples were collected on days 1, 35, and 84. Filtrates from samples were collected to determine microbial biomass and available N. Available N samples were extracted with 0.5 M $K_2SO_4$ solutions at a 1:5 (w:v) soil to extract ratio and shaken at 250 rpm on a mechanical shaker for 1 hour then filtered using Whatman No. 42 filter paper (Norman and Bremner, 1965). Nitrate-N and $NH_4^+$-N were determined colorimetrically using vanadium (III) in acid and indophenol blue methods, respectively (Norman and Bremner, 1965; Miranda et al., 2001). Soil samples were dried at 70 °C and ground (<2 mm) with a mortar and pestle for total C and N measurements, which were determined using dry combustion techniques with an automated CN analyzer (Carlo Erba, Milan, Italy).

### *3.2.5 Statistical Analysis*

All statistical calculations were performed using R software version 1.1.456 (R Foundation for Statistical Computing, Vienna, Austria) with statistical significance set at $\alpha=0.05$ for all tests. Two-way analysis of variance (ANOVA) was used to analyze the effect of soil type, organic amendment, and their interaction on cumulative GHG emissions and biogeochemical properties throughout the entire incubation.

Normality of distribution and homogeneity of variance of the data were confirmed with Shapiro and Bartlett tests. Non-homogeneous data of anthropogenic GHG emissions, $CH_4$ emissions, $NH_4^+$-N, and C/N ratio were adjusted with first order auto-regression AR(1) and stratum variance and $N_2O$ emissions were adjusted with first order auto-regression AR(1). Cumulative GHG emissions and initial soil and manure type properties were analyzed using a Tukey-Kramer test. Relationships between initial soil properties and cumulative GHG emissions were examined using Spearman's rank correlation.

## **3.3 Results & Discussion**

### *3.3.1 $CO_2$ Emissions*

There was an initial peak in $CO_2$ emissions (Fig. 3.3a) from the addition of cattle manure at the beginning of the incubation period. Carbon dioxide emissions are a product of microbial activity in the soil due to the improved availability of substrate C to stimulate microbial activity as well as the direct addition of microorganisms from the manure (Hadas and Portnoy, 1994; Ros et al., 2006; Reeve et al., 2012). At day 35, there was another peak similar to the rates observed for day 7 (Fig. 3.3a). This peak was prompted by the change from 60% to 80% water-filled pore space (Smith et al., 2003). As water-filled pore space increases, decomposition can increase for a short period because a second accumulation of OM occurs and corresponding $CO_2$ emissions are released (Ruser et al., 2006; Weil and Brady, 2017). Similar to Hadas and Portnoy (1994) and Hao et al. (2004), $CO_2$ emissions declined over time (Fig. 3.3a) from the suspected decline in OM availability and consequent reduction in microbial activity and corresponding $CO_2$ emissions.

Diet type had a significant impact on $CO_2$ emissions, as the BM-amended soils had the significantly greatest cumulative $CO_2$ emissions across OBC and OGL soil types (Fig. 3.4a). The difference likely comes from the significantly higher C/N ratio present in the BM manure, potentially promoting mineralization of OC, which released greater $CO_2$ emissions (Table 3.1) (Calderón et al., 2004; van Vliet et al., 2007). However, our Pearson's correlation coefficient indicates a lack of significant relationship between C/N ratio and $CO_2$ emissions, although there was a positive relationship between $CO_2$ emissions and available N (Table 3.4). Weier (1993) found increased C was correlated with lower $NO_3^-$-N ($NO_3^-$-N is lost due to increased denitrification), which might explain why BM soil had increased C/N ratio and $CO_2$ emissions.

I found that both the BM-amended soil had significantly greater $CO_2$ emissions than 3NOPC-amended soils across OBC and OGL soil types (Fig. 3.4a). The composting process allows for mixing of the manure and exposure of more surface area to undergo aerobic, rather than anaerobic, decomposition (Hao et al., 2001). This results in a highly recalcitrant organic fertilizer resistant to microbial breakdown when applied to soil (Eldor, 2015; Weil and Brady, 2017). The presence of $O_2$ oxidation of labile C creates functional chemical groups that become charged when they dissociate, increasing its solubility (Weil and Brady, 2017). The polarity change transforms many hydrophobic nonpolar biomolecules into hydrophilic molecules that are more soluble, allowing for greater microbial activity and $CO_2$ emissions during the composting process (Hao et al., 2001; Ros et al., 2006; Li et al., 2016a; Weil and Brady, 2017). The surge of $CO_2$ emissions during composting means less $CO_2$ emissions would be expected from the final composted product. Humification resulted in lower C/N ratios in 3NOPC (Table 3.1), which would be expected to reduce microbial activity potential and release of $CO_2$ emissions upon application to the soil (Li et al., 2016a).

The manure and soil types showed a statistically significant interaction for $CO_2$ emissions (Table 3.4). Greater $CO_2$ emission rates were noted in OGL soil, with lower emission rates in OBC and DBC soils (Fig. 3.4a). Similar to Li et al. (2016a), $CO_2$ emission rates were highly correlated with pH. The pH was significantly lowest in OGL soils and significantly different across manure types: the highest pH was for BM, then 3NOPM, and the lowest 3NOPC (Table 3.1). With the addition of the manure and compost, a larger change in pH occurred in the OGL soil because the DBC and OBC soils had a pH closer to the pH of the manure added (Weil and Brady, 2017).

The final pHs of the amended-soils were 7.66, 7.70, and 7.76 for BM, 3NOPM, and 3NOPC amended DBC, 7.22, 7.13, 7.14 for BM, 3NOPM, and 3NOPC amended OBC, 6.29, 5.61, 6.26 BM, 3NOPM, and 3NOPC amended OGL respectively. The greater change in pH for OGL soils may have led to increased solubilization of OC, microbial activity, and $CO_2$ emissions (Li et al., 2016a; Weil and Brady, 2017). The relationship is demonstrated by the negative correlation between pH and $CO_2$ emissions, thus a lower soil pH, when amended with manure, suggests higher $CO_2$ emissions (Table 3.4). Finally, the DBC soil showed no statistical difference between treatments and between amended and non-amended soils. The neutral pH of the DBC soil coupled with the statistically lower available N (Table 3.1) may have lowered microbial activity and limited the effects of the amendments (Weil and Brady, 2017).

### *3.3.2 $N_2O$ Emissions*

Similar to $CO_2$ emissions, an initial peak in $N_2O$ emissions from the addition of organic amendments occurred at the beginning of the incubation (Fig. 3.3b) from the growing microbial activity (Bremner and Shaw, 1958; Hao et al., 2004; Ruser et al., 2006). The $N_2O$ emissions from most soil and management types peaked on day one. The initial $N_2O$ emissions probably came from nitrification, which occurs under aerobic conditions (<60% water-filled pore space) (Paustian et al., 1992; Smith et al., 2003; Eldor, 2015; Weil and Brady, 2017).

The initial increase in $N_2O$ emissions continued until day 42 (Fig. 3.3b) This extended release of $N_2O$ emissions may have occurred from the change to 80% water-filled pore space, which likely allowed for denitrification (Ruser et al., 2006). Without anaerobic conditions, the $N_2O$ emissions would only be present for the first few weeks as observed by Li et al. (2016a) and Bhandral et al. (2007). Denitrification occurs under anaerobic conditions (>60% water-filled pore space) when the lack of $O_2$ requires denitrifying microorganisms to utilize $NO_3^-$-N instead of $O_2$ (Bremner and Shaw, 1958; Smith et al., 2003; Datta et al., 2017; Weil and Brady, 2017). Nitrous oxide emissions then slowed down considerably at day 49 until the end of the incubation (Fig. 3.3b) (Smith et al., 2003; Li et al., 2016a).

Diet type had a significant impact on $N_2O$ emissions, as the 3NOPM-amended soils had greater $N_2O$ emissions than the BM-amended soils, except in OGL soils (Fig. 3.4b). Nitrate is an essential factor in denitrification rates, and increasing $NO_3^-$-N means greater rates of $N_2O$ emissions from anaerobic processes (Paustian et al., 1992; Hao et al., 2004; Ruser et al., 2006). In the rumen, the addition of 3-NOP supplement increases $NO_3^-$-N by placing its reducible $NO_3^-$-N group to oxidize Ni (Datta et al., 2017). This could explain the significantly greater $NO_3^-$-N in 3NOPM and 3NOPC.

The OGL soil had statistically similar $N_2O$ emissions from BM-amendment and 3NOPM-amendment in comparison to OBC and DBC soils (Fig. 3.4b). The interaction of soil and manure type was highly significant for $N_2O$ emissions (Table 3.4), which may be attributable to the higher C/N ratio in BM interacting with the higher $NH_4^+$-N in OGL (Table 3.1). Higher C can enhance denitrification by directly providing donor electrons and stimulating $O_2$ consumption, and low levels of $NH_4^+$-N can inhibit microbial assimilatory $NO_3^-$-N reduction (Paustian et al., 1992; Eldor, 2015). Together, the ideal environment is provided for denitrification to occur and release $N_2O$ emissions. Moreover, air-drying soil and rewetting it can cause 2-10% more total C, so this must be considered when comparing the results of this incubation to field studies (Kaiser et al., 2015).

Nitrous oxide emissions were significantly lower in 3NOPC than in 3NOPM amendments across all soil types (Fig. 3.4b). Because of the soil moisture reductions that occur during composting, a large flux from 3NOPC-amended soils did not occur until later in the incubation (Fig. 3.3b). The higher water content in 3NOPM means lower $O_2$ availability and greater labile C upon initial application (Gregorich et al., 2005; Ruser et al., 2006). The water content results in higher OC and C/N ratio of the 3NOPM than 3NOPC (Table 3.1). Because the 3NOPC already experienced microbial breakdown through composting, less readily decomposable material in

3NOPC may have slowed the rates of decomposition, and thus $N_2O$ emissions were lower (Bremner and Shaw, 1958).

All CK-amended soils saw a rise in $N_2O$ emissions (Fig. 3.3b), from the increased WFPS which may have prompted denitrification (Hao et al., 2001). However, soils amended with BM and 3NOPM saw a continued decline in $N_2O$ emissions, as the amendment is already high in water content (Reeve et al., 2012). The lack of air-filled pores means low $O_2$ concentrations, therefore $N_2O$ may have continued denitrification to a further stage, producing $N_2$ (Paustian et al., 1992; Hao et al., 2001).

Soil type had a significant impact on $N_2O$ emissions, as the OBC soil had the lowest $N_2O$ emissions (Fig. 3.4b). The sandy loam texture of the OBC soils in comparison to the clay loam textures of the OGL and DBC soils matches the Paustian et al. (1992) observation that $N_2O$ emissions increase for soils with smaller particle sizes. The smaller particle size allows for more water retention, so as WFPS increases, the rate of nitrification and $N_2O$ emissions may have increased (Smith et al., 2003). There is a strong correlation between available N ($NH_4^+$-N, and $NO_3^-$-N) and $N_2O$ emissions (Table 3.4). The OGL soils also had significantly higher available N, $NH_4^+$-N, and $NO_3^-$-N than the DBC and OBC soils for all treatments (Fig. 3.2), which may have resulted in greater microbial activity throughout the incubation, and N loss through $N_2O$ emissions (Smith et al., 2003).

Additionally. the potential cause for the different $N_2O$ emission trends between OGL, DBC, and OBC soils was the effect of different initial pH. The neutral-basic OBC and DBC soils started with very little $NH_4^+$-N, so it was likely that $NH_4^+$-N was quickly used during nitrification for $NO_3^-$-N production (Weil and Brady, 2017). The OBC and DBC soils may have been limited to denitrification afterward, as there was not enough $NH_4^+$-N available for nitrification (Fig. 3.2a).

However, acidic soils produce more $NH_4^+$-N under waterlogged conditions, as seen through the higher $NH_4^+$-N in OGL soils throughout the experiment (Bremner and Shaw, 1958; Paustian et al., 1992). Therefore, the OGL soil may have both nitrification and denitrification occurring simultaneously that contributed to $N_2O$ emission. Despite there being anaerobic conditions in the latter half of the incubation, the OGL soils may have also exhibited anammox, the anaerobic oxidation of $NH_4^+$-N ions, as demonstrated by the increase in $NO_3^-$-N towards the end of the incubation (Fig. 3.2b) (Eldor, 2015; Weil and Brady, 2017)..

### *3.3.3 $CH_4$ Emissions*

The $CH_4$ emissions from all soil and management types peaked on various days, but most fluxes occurred before day 35 (Fig. 3.3c and 3.3d). This observation is similar to the Hao et al. (2004) finding that less than 50% of total $CH_4$ emission fluxes occurred during the first 28 days. Le Mer and Roger (2001) observed a positive correlation between methanogenic potential and OM. Methane emissions increase when total C increases, as a high C/N ratio organic material is rich in labile C (Le Mer and Roger, 2001; Hao et al., 2004). The C/N was found to be significantly correlated to $CH_4$ emissions (Table 3.4). Methane emissions are produced when OM is mineralized in anaerobic environments with low redox potential (Le Mer and Roger, 2001; Smith et al., 2003).

Addition of the manure may have also created a temporarily anaerobic zone in the soil surface, enhancing and stabilizing the initial $CH_4$ production (Hütsch et al., 1993b; Hao et al., 2004). After day 21, most $CH_4$ emissions were negative, suggesting that the manure and soil had become sinks. A decline in $CH_4$ emissions is observed as the nutrients from the manure amendment likely depleted (Mor et al., 2006). An environment is considered a $CH_4$ source when the sum of the production of methanogenic bacteria and consumption by methanotrophic bacteria are positive and considered a $CH_4$ sink when the sum is negative and reacts with $OH^-$ radicals (Hütsch et al., 1993a; Le Mer and Roger, 2001; Gregorich et al., 2005).

Manure type and manure and soil type interaction both did not have a significant impact on $CH_4$ emissions (Table 3.3), so soil type and manure type will be discussed separately. There were no statistical differences between the manure treatments, and all treatments resulted in negative $CH_4$ emissions except 3NOPM (Fig. 3.4d). The quality of OM plays an important role, with readily decomposable organic compounds inducing rapid denitrification (Bremner and Shaw, 1958; Paustian et al., 1992). The OC is highest for 3NOPM, followed by BM, then 3NOPC (Table 3.1). The higher OC in 3NOPM may mean greater microbial activity and the resulting positive $CH_4$ emissions (Hadas and Portnoy, 1994; Larney and Hao, 2007). However, these effects are almost negligible, as the numbers are extremely small and statistically similar, so further research should focus on the importance of soil type when looking at $CH_4$ emission.

The OBC soils displayed statistically different and negative $CH_4$ emissions in all amendment types, affirming that their properties lead them to serve as a $CH_4$ sink (Fig. 3.4c) (Le Mer and Roger, 2001). The OBC soils had the lowest C/N (Table 3.1), which may have limited some microbial activity (Le Mer and Roger, 2001; Hao et al., 2004; Wang et al., 2012). Smaller soil particle size (as DBC and OGL soils are clay loams compared to the OBC sandy loam soil) is also expected to decrease $CH_4$ emissions due to poor drainage and anaerobic conditions (Le Mer and Roger, 2001).

The higher ability to retain water in OGL and DBC soils promotes methanogen transport as they are planktonic, allowing them to move from the manure surfaces to the soil surfaces (Staley et al., 2011; Eldor, 2015). Furthermore, high silt and clay content can protect OM from mineralization and favor trapping $CH_4$ bubbles in soils (Le Mer and Roger, 2001). Finally, caution must be applied when utilizing this data in the field. The rates of $CH_4$ emissions in the field will be higher than those calculated in the laboratory, as the $CH_4$ emissions measured in the headspace do not account for $CH_4$ produced in the soil (Hütsch et al., 1993b).

## 3.4 Conclusions

Our results determined that the anthropogenic GHG emissions (expressed as $CO_2$ equivalent) for composted manure from cattle fed 3NOP additive was lowest when applied to soil (Fig. 3.4e), meaning that 3NOP additive should be considered as a viable amendment to manure that will be composted. Manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected $CO_2$ and $N_2O$ emissions, while only soil type affected $CH_4$ emissions. The diet management of 3NOP amendment to cattle feeds affected the properties of cattle manure that influenced the chemical and physical properties upon the amendment to different soil types and the resulting GHG emissions. Composted manure resulted in lower GHG emissions across all soil types from stabilized C and N that limited additional microbial activity when applied to soil. Addition of 3NOP to the cattle feed increased available N in the manure. The use of 3NOP led to reduced $CO_2$ emissions and increased $N_2O$ emissions. The results from this laboratory incubation are limited in scope because rewetting air-dried soils under field conditions can alter the soil properties. Additionally, due to the variability in composts, it is important to test the properties of the amendments before applying them to the field. Supplementary emissions management of manure such as stockpiling should also be considered.

## 3.5 Tables and Figures

**Table 3.1.** Chemical properties of soil and manure types used in incubation (means ±SE) ($n = 3$).

| Parameter | pH* | TN (g kg$^{-1}$) | OC (g kg$^{-1}$) | C/N ratio | NH$_4^+$-N (mg kg$^{-1}$) | NO$_3^-$-N (mg kg$^{-1}$) | AN (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Manure Type | | | | | | | |
| BM | $7.39 \pm 0.05^{a}$ | $10.4 \pm 0.21^{b}$ | $100 \pm 2.13^{a}$ | $9.58 \pm 0.02^{a}$ | $7.53 \pm 1.04^{a}$ | $635 \pm 13.0^{b}$ | $643 \pm 19.4^{a}$ |
| 3NOPM | $7.09 \pm 0.02^{b}$ | $12.8 \pm 0.51^{a}$ | $114 \pm 4.09^{b}$ | $8.89 \pm 0.09^{b}$ | $10.9 \pm 1.04^{a}$ | $1098 \pm 32.^{a}$ | $1109 \pm 30.7^{b}$ |
| 3NOPC | $6.99 \pm 0.03^{c}$ | $9.62 \pm 0.08^{b}$ | $85.1 \pm 2.25^{c}$ | $8.84 \pm 0.17^{b}$ | $11.9 \pm 0.60^{a}$ | $1056 \pm 16.2^{a}$ | $1068 \pm 22.0^{b}$ |
| Soil Type | | | | | | | |
| DBC | $7.31 \pm 0.03^{a}$ | $1.97 \pm 0.29^{ab}$ | $22.0 \pm 4.39^{a}$ | $14.3 \pm 0.13^{a}$ | $3.00 \pm 0.33^{b}$ | $6.45 \pm 0.02^{a}$ | $9.03 \pm 0.45^{a}$ |
| OBC | $7.25 \pm 0.06^{a}$ | $2.44 \pm 0.39^{a}$ | $23.0 \pm 4.12^{a}$ | $9.36 \pm 0.35^{b}$ | $4.02 \pm 0.47^{b}$ | $31.8 \pm 1.18^{a}$ | $35.9 \pm 1.48^{b}$ |
| OGL | $3.91 \pm 0.04^{b}$ | $0.98 \pm 0.14^{b}$ | $12.2 \pm 2.53^{a}$ | $12.0 \pm 1.05^{a}$ | $6.47 \pm 0.23^{a}$ | $49.4 \pm 1.64^{a}$ | $55.0 \pm 1.43^{c}$ |

**Note**: Soil type: DBC, Dark Brown Chernozem; OBC, Orthic Black Chernozem; OGL, Orthic Gray Luvisol. Manure type: BM, barley-based manure; 3NOPM, manure from cows fed 3NOP supplement; 3NOPC, composed manure from cows fed 3NOP supplements. Abbreviations: AN, Sum of NH$_4^+$-N and NO$_3^-$-N; OC, organic carbon; TN, Total nitrogen; *Soil pH was measured in 1:5 soil:water ratio.

**Table 3.2.** Results of multi-way ANOVAs (p-values) testing the effects of soil type (S), manure type (M), time (T) and their interactions on soil biogeochemical properties (*n* = 4).

| Source of Variation | S | M | T | S x M | S x T | M x T | S x M x T |
|---|---|---|---|---|---|---|---|
| $NH_4^+$-N | < 0.001 | 0.883 | 0.287 | 0.322 | 0.210 | < 0.001 | < 0.001 |
| $NO_3^-$-N | < 0.001 | < 0.001 | 0.097 | 0.065 | 0.179 | < 0.001 | 0.320 |
| AN | < 0.001 | < 0.001 | 0.110 | 0.364 | 0.290 | < 0.001 | 0.502 |
| TN | < 0.001 | < 0.001 | 0.424 | 0.167 | 0.771 | 0.231 | 0.101 |
| C/N Ratio | < 0.001 | < 0.001 | 0.122 | < 0.001 | 0.047 | 0.979 | 1.000 |
| OC | < 0.001 | < 0.001 | 0.357 | 0.413 | 0.659 | 0.258 | 0.163 |

Abbreviations: AN, available nitrogen; TN, total nitrogen; OC, organic carbon.

**Table 3.3.** Results of two-way ANOVAs (p-values) testing the effects of manure type (M), soil type (S), and their interactions (S x M) on cumulative GHG emissions (*n* = 4).

| Source of Variation | S | M | S x M |
|---|---|---|---|
| $CO_2$ Emissions | $< 0.001$ | $< 0.001$ | $< 0.001$ |
| $N_2O$ Emissions | $< 0.001$ | $< 0.001$ | $< 0.001$ |
| $CH_4$ Emissions | $< 0.001$ | 0.368 | 0.265 |
| Anthropogenic GHG Emissions | $< 0.001$ | $< 0.001$ | $< 0.001$ |

**Table 3.4.** Pearson's correlation coefficients of cumulative $CO_2$, $N_2O$, and $CH_4$ emissions to initial soil properties (*n* = 12).

| Variable | pH | OC | TN | C/N | $NH_4^+$-N | $NO_3^-$-N | AN |
|---|---|---|---|---|---|---|---|
| $CO_2$ | -0.60** | -0.17 | -0.29 | 0.07 | 0.59** | 0.37* | 0.38* |
| $N_2O$ | -0.61** | -0.15 | -0.27 | 0.06 | 0.59** | 0.35* | 0.36** |
| $CH_4$ | -0.04 | -0.15 | -0.25 | 0.44** | -0.03 | 0.07 | 0.06 |

Abbreviations: AN, available nitrogen; TN, total nitrogen; OC, organic carbon. *, $P < 0.05$; **, $P < 0.01$.

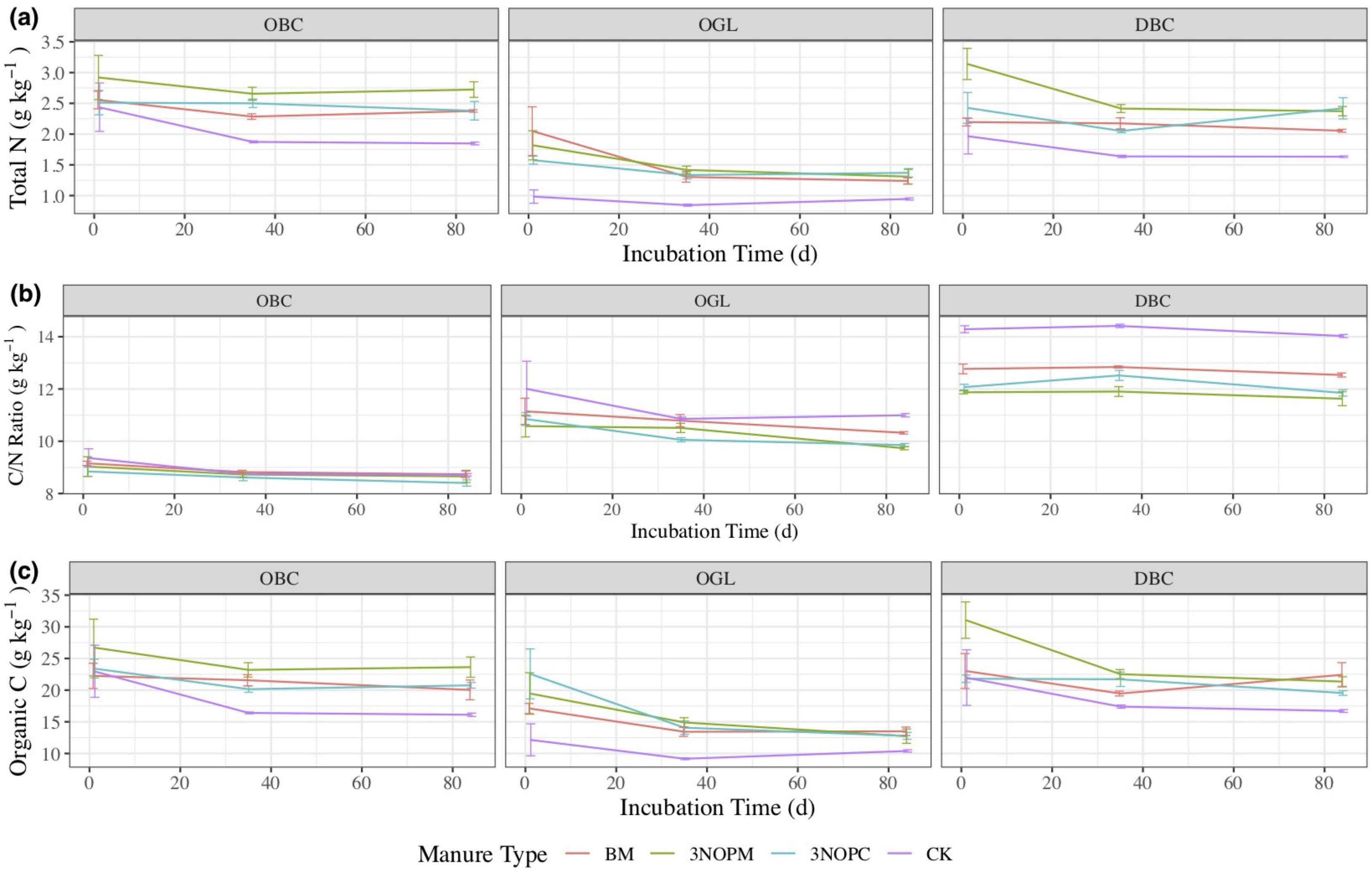

**Fig. 3.1** Effects of manure type on (a) total nitrogen, (b) carbon/nitrogen ratio, and (c) organic carbon in various soil types throughout the incubation. Soil type: DBC, Dark Brown Chernozem; OBC, Orthic Black Chernozem; OGL, Orthic Gray Luvisol. Manure type: BM, barley-based manure; 3NOPM, same as BM manure, except with 200 mg kg$^{-1}$ 3-NOP supplement; 3NOPC, compost produced using 3NOPM. CK, control with no amendments. Vertical bars indicate standard errors of the means (*n* = 4).

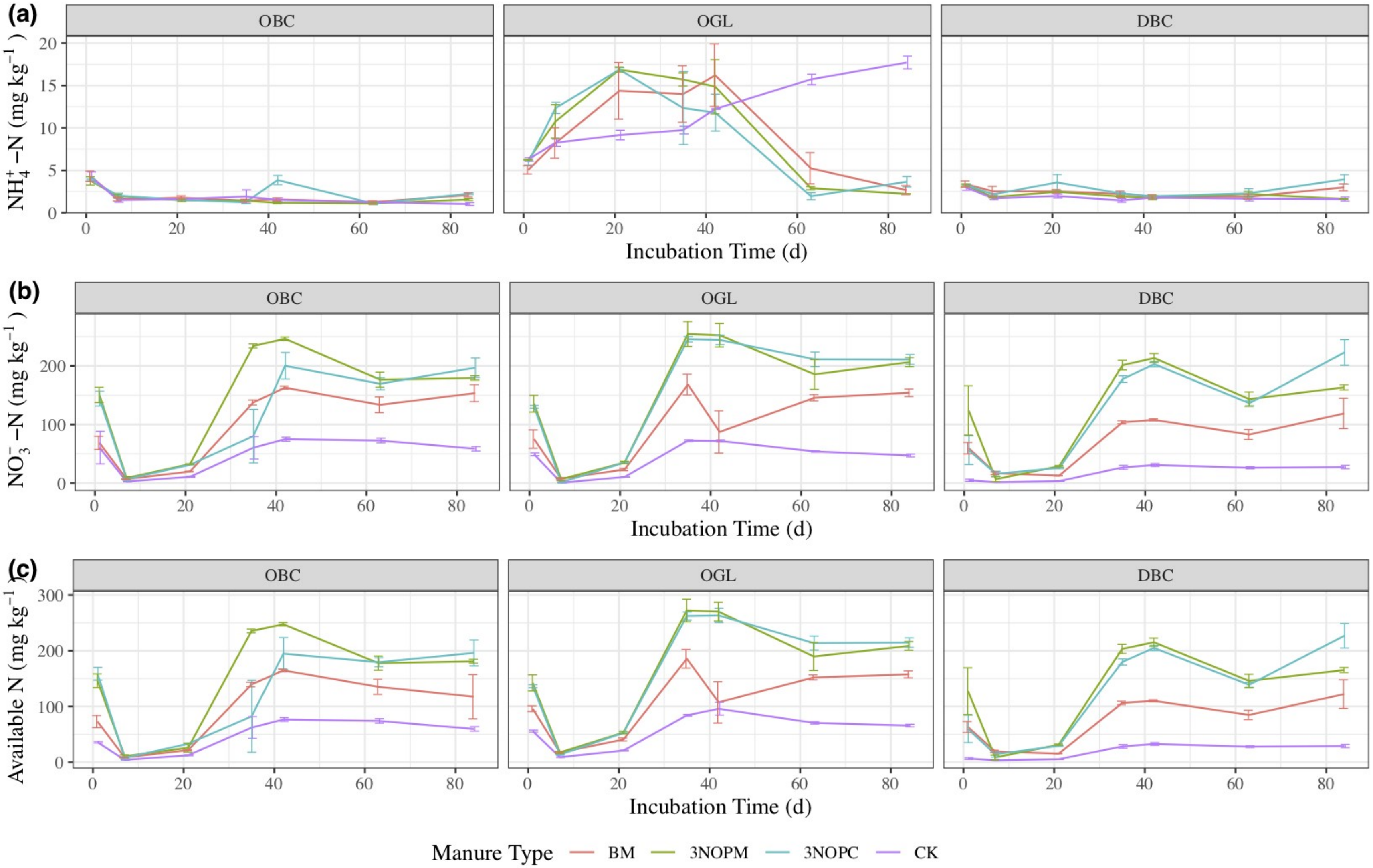

**Fig. 3.2** Effects of manure type on (a) ammonium, (b) nitrate, and (c) available nitrogen in various soil types throughout the incubation. Soil type and manure type codes are identical to Fig 3.1. Vertical bars indicate standard errors of the means (*n* = 4).

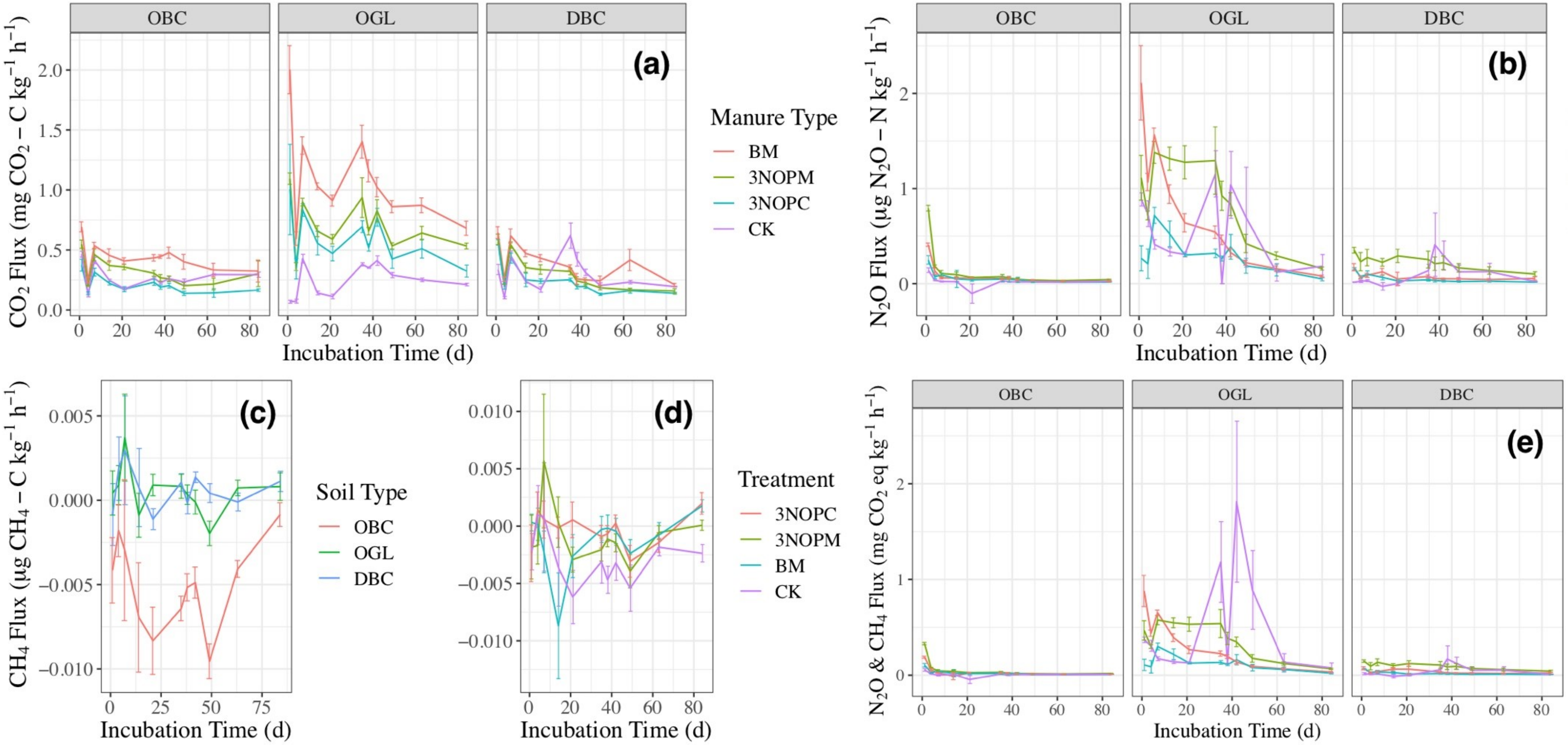

**Fig. 3.3** Effects of manure on (a) $CO_2$, (b) $N_2O$, (c) and (d) $CH_4$, and (e) Anthropogenic GHG emissions in various soil types throughout the incubation. Soil type and manure type codes are identical to Fig 3.1. Vertical bars indicate standard errors of the means (*n* = 4).

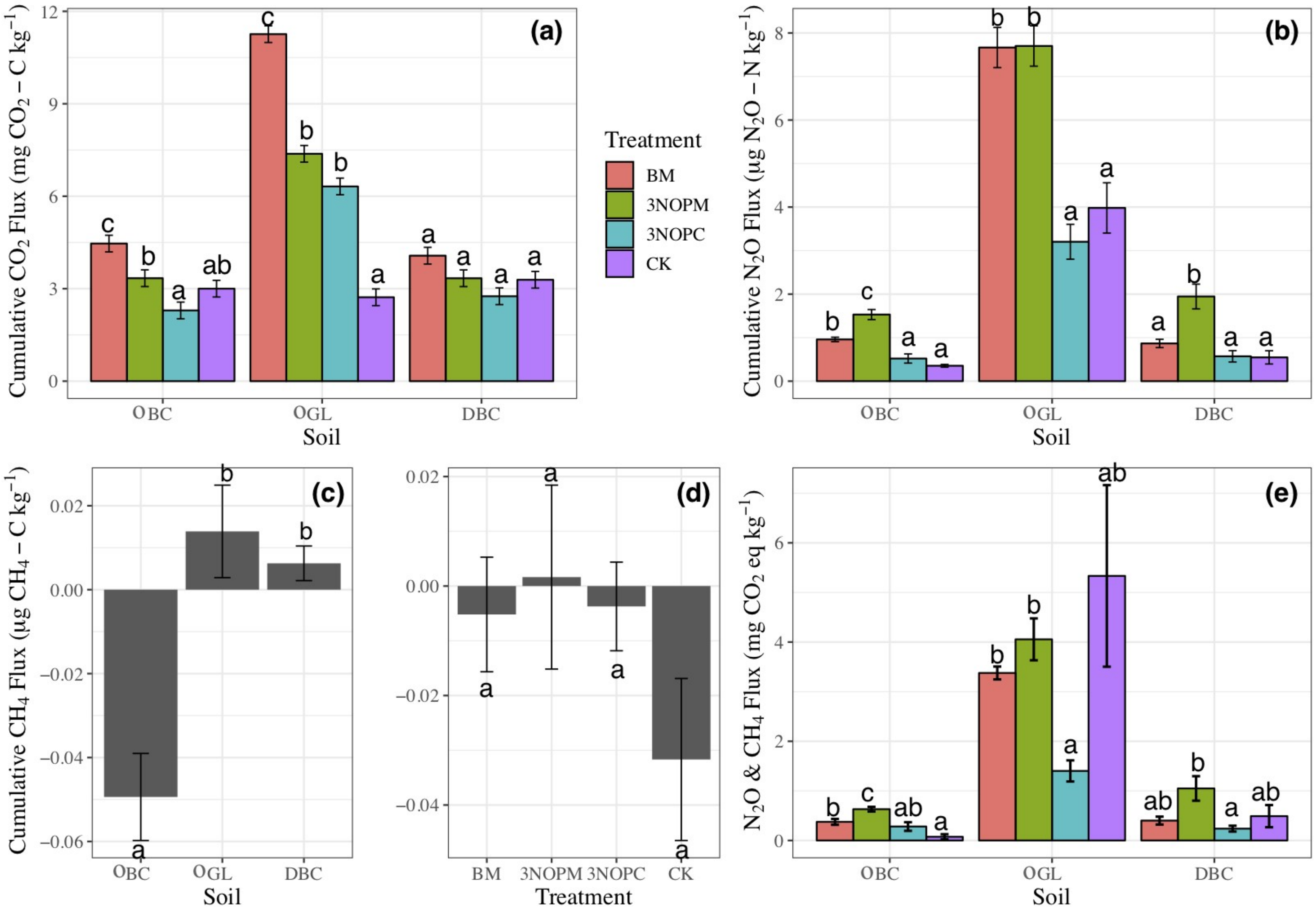

**Fig. 3.4** Effects of manure on cumulative (a) $CO_2$, (b) $N_2O$, (c) and (d) $CH_4$, and (e) Anthropogenic GHG emissions in various soil types. Soil type and manure type codes are identical to Fig 3.1. Letters denote significant differences between treatments, treatments that do not share the same letter are significantly different from each other. Vertical bars indicate standard errors of the means (*n* = 4).

# Chapter 4. Synthesis, Conclusions, and Future Research

## 4.1 Synthesis of Research Findings

### *4.1.1 Objectives*

This thesis was targeted at reducing greenhouse gas emissions in an incubation setting. The two experiments allowed comparison of emissions from the manure of cattle fed various feed compositions and additives when that manure was added as an organic amendment to different soil types. The two main questions addressed were:

1. *How do changes to cattle feed affect the resulting manure properties, and how does this affect soil properties when it is applied as an organic amendment?*
2. *How do these changes in soil properties influence the release of natural ($CO_2$) and anthropogenic ($N_2O$ and $CH_4$) GHG emissions?*

This chapter summarizes the key findings using controlled incubation experiments with specific soil types.

### *4.1.2 $CO_2$ Emissions*

Overall, manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected $CO_2$ emissions. Increased $CO_2$ emissions occurred as a result of high C/N ratio and corresponding microbial respiration (Calderón et al., 2004; van Vliet et al., 2007). The 3NOPM- and BM-amended soil had greater emissions than 3NOPC-amended soils across all soil types as composting lowered C/N ratios in the second experiment which reduces microbial activity potential

If there are statistically similar C/N ratios, as in experiment one, the stressors on the microbes become the determining factor. The pH was significantly higher in BM, in the first experiment suggesting that the microbes face increased stress in acidic environments and released greater $CO_2$ emissions (Domsch and Anderson, 1993). Similarly, the change in pH when the manure was added could explain the difference in $CO_2$ emissions between soil types (Sadeghpour et al., 2016; Curtin et al., 2016; Li et al., 2016a). A greater change in pH from soils with a lower pH was found to have increased solubilization of OC, allowing for enhanced microbial activity and $CO_2$ emissions were found in both experiments.

The $CO_2$ emissions of experiment one were almost 10 times the values of experiment two. This may come from the age of the manure used in the two incubations. The manure from the first experiment was collected during winter 2014 and spring 2015 when the cattle were on backgrounding diets (Chibisa and Beauchemin, 2018). As it was collected directly from the cattle's pens, the manure was fresh and free of bedding material or soil particles. In contrast, the manure from the second experiment was collected during autumn 2016 and underwent composting or stockpiling during winter 2016 (Vyas et al., 2018). The experiment was conducted from winter 2015 to fall 2016 where the manure was mixed with bedding materials and soil particles.

As the manure from the second experiment was 2-2.5 years old from excretion to collection in comparison to the first experiment, so many of the microbial interactions may have long ended in comparison to the manure used in the first incubation (Hashimoto et al., 1981; Hao et al., 2001; Ros et al., 2006). Because of this, the pH of the amendments was higher for the first experiment (Table 2.1) than the second experiment (Table 3.1). The greater change in pH induced by the manure amendment resulted in increased solubilization of OC, microbial activity, and $CO_2$ emissions (Li et al., 2016a; Weil and Brady, 2017). Additionally, the OC and C/N of the first experiment was almost double (Table 2.1) of the second experiment (Table 3.1) from the quicker utilization of fresh manure. Therefore, microbes had much greater labile C for their reactions and respiration (Weil and Brady, 2017).

### *4.1.3 $N_2O$ Emissions*

Overall, manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected $N_2O$ emissions. These differences in $N_2O$ emissions may have resulted from differences in the form of N excreted from animals fed the respective diets, which altered the $NH_4^+$-N/ $NO_3^-$-N ratio (Ørskov, 1986; Chibisa and Beauchemin, 2018). The soils amended with BM had the highest emissions, attributing the difference in $NH_4^+$-N values between the CM and BM (experiment one). The 3NOPM-amended soils had greater emissions than the BM-amended soils, except in OGL soils (experiment two). This may be because the addition of 3-NOP increased $NO_3^-$-N as it acted as a chemical analog to oxidize Ni (Duin et al., 2016). Furthermore, in the second experiment, low levels of $NH_4^+$-N inhibited microbial assimilatory and $N_2O$ emissions in OGL soils (Paustian et al., 1992; Eldor, 2015).

The difference in $N_2O$ emissions between BM- and CM-amended soils was not significant for OBC and OGL soils (experiment one) and the OBC soil had the lowest $N_2O$ emissions (experiment two). In the second experiment, the OGL and DBC are both clay loam soils, in comparison to the silty clay OBC. The greater clay percentage and smaller pore spaces contributed to the greater emissions from enhanced water retention and denitrification which produce $N_2O$ emissions (Weier et al., 1993; Van Zwieten et al., 2010). Furthermore, less readily decomposable material and lower water retention in 3NOPC may have slowed the rates of decomposition and $N_2O$ emissions (Bremner and Shaw, 1958).

Once again, the $N_2O$ emissions of experiment one (Fig 2.4) much larger than the values of experiment two (Fig 3.4). The $NH_4^+$-N levels of the first experiment (Table 2.1) were nearly 5 times of the second experiment (Table 3.1); however, there was no $NO_3^-$-N present in the first experiment. The delayed collection of the manure suggests that $NH_4^+$-N may have been transformed into $NO_3^-$-N (Weil and Brady, 2017). The $NO_3^-$-N is less subjective to GHG losses than $NH_4^+$-N, explaining why we see more $NO_3^-$-N and lower $N_2O$ emissions in the second experiment.

### *4.1.4 $CH_4$ Emissions*

Overall, manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected $CH_4$ emissions in experiment one, but only soil type was statistically significant in experiment two. The pH plays a large role in determining $CH_4$ emissions as it is a driver for the microbial reactions responsible for $CH_4$ production. For the first experiment, $CH_4$ emissions were highest for BM-amended soils in OBC and OGL soil types. as starch from corn is less digestible in the rumen, resulting in greater hind-gut fermentation and lower fecal pH (Ørskov, 1986; Johnson et al., 2000; Gibb and McAllister, 2003; Chibisa and Beauchemin, 2018). Similarly, the DBC soil in the first project had significantly higher pH than the other soils, suggesting the potential to promote specialized methanogens that are isolated in the rumen that may play a role in only certain soil pH ranges (Boadi et al., 2004; Sampanpanish, 2012).

Because $CH_4$ production occurs under anaerobic conditions, the $H_2O$ and $O_2$ content in the soil was another primary driver of $CH_4$ emission levels. The OBC soils in experiment two had the highest $CH_4$ emissions as a result of smaller soil particle size, which leads to poor drainage as well as protecting OM from mineralization and favors trapping $CH_4$ bubbles in soil (Le Mer and Roger, 2001; Hao et al., 2004; Wang et al., 2012).

Finally, there were great differences between $CH_4$ emissions between the two experiments. The first experiment had all positive $CH_4$ emissions (Fig 2.4), and the second experiment had negative emissions primarily at the beginning of the experiment but continued throughout (Fig 3.4c and 3.4d). Moreover, the emissions from the first experiment were 100x greater than the second experiment, a much greater difference than $CO_2$ and $N_2O$ emissions.

The difference may have come from the composition of the manure applied in each experiment. The manure from the first experiment was fresh manure free of bedding material or soil particles were collected (Chibisa and Beauchemin, 2018), while experiment two manure was mixed with bedding materials and soil particles (Vyas et al., 2018). Hashimoto (1981) found that a greater degree of contamination of the manure also lowers $CH_4$ emissions, as the straw and other particles may have increased $O_2$ access to the soil particles. More aerobic conditions would explain the negative fluxes present in the second experiment.

## 4.2 Implications for Future Research

### *4.2.1 Analyzing Economic Considerations*

Our findings leave many future questions to be investigated. Our experiments only analyzed the environmental impacts, with no consideration of the practicality for farmers, especially in regard to economics. Further work should investigate the net cost of barley or corn as feed and the additional expenses of 3-NOP, as the cheaper option will almost always be preferred if the benefits do not outweigh the costs (Gibb and McAllister, 2003; Liebig et al., 2005). Corn feed contains higher levels of starch making digestion of corn more difficult than barley for cattle (Johnson et al., 2000; Gibb and McAllister, 2003). Therefore, cattle require more corn feed to provide equal nutritional benefits as barley feed. Many other factors can change the price of corn and barley, such as dry rolling barley. This can increase the nutrients available to the cattle by 15%, which would make it a cheaper and more efficient feed than corn (Gibb and McAllister, 2003). Even if one feed is more sustainable, the practical applications need to be further observed.

### *4.2.2 Looking at Variability in Soil and Manure Types & Interactions*

Due to the variability in each treatment studied, it is important to test the chemical properties of the amendments prior, as the results may differ from this thesis. For example, active composting with forced aeration may result in different GHG emissions from manure-amended soil than passive composting (Larney and Hao, 2007; Jayasundara et al., 2016). The time at which the manure completes the composting process can alter the C/N ratio and organic matter in the final product, and thus the microbial respiration rates (Hadas and Portnoy, 1994; Larney and Hao, 2007). Additionally, the environmental conditions of the manure can influence the microbial population and GHG emissions, as the aerobic microbial community can go into cold shock during cold season composting (Jayasundara et al., 2016). By looking at different environments, further insight may be gained into how the microbial biomass N in manure interacts with the soil (Hadas and Portnoy, 1994; Ros et al., 2006).

### *4.2.3 Investigating Additional Soil Orders*

For both experiments, manure type, soil type, and their interaction significantly ($\alpha = 0.05$) affected GHG emissions. This was primarily found to be due to different soil texture compositions that can change conditions from aerobic to anaerobic because smaller pore size leads to greater water retention (Weier et al., 1993; Le Mer and Roger, 2001). Therefore, future work should investigate the effect of additional soil types on GHG emissions in the field following manure application. Additionally, Boadi et al. (2004) suggest that there are specialized methanogens that are isolated in the rumen that may play a role in only certain soil pH ranges. There could be other microbial interacts that were not present in these experiments that would alter the C and N cycles and GHG emissions.

### *4.2.4 Understanding the Extent of Rewetting Effects*

The results from these laboratory incubations are limited in scope because rewetting air-dried soils under field conditions may change microbial functions. The “Birch Effect”, the rewetting of air-dried soil, can cause a burst of $CO_2$ emissions (Kaiser et al., 2015). This occurs from the mineralization of intracellular solutes and the resulting increase in assimilable C supply (Kaiser et al., 2015). While our preincubation period should have accounted for this effect, the phenomenon has been known to persist for several days so the exact impact of its effects should be analyzed to understand $CO_2$ emissions in field conditions or using fresh soils (Kaiser et al., 2015). The extent of the Birch Effect is also variable upon each soil type, as some soils are subject to a wet and dry season, resulting microorganisms adapted to rewetting (Ruser et al., 2006). Soil scientists should consider all environmental aspects that will alter soil dynamics.

### *4.2.5 Exploring the Life Cycle Analysis*

Additional emissions management of manure such as stockpiling, transporting of manure, etc. should also be considered. Even though feeding cattle a corn- rather than barley-based diet and compost from cattle fed 3-NOP may help reduce the total GHG emissions from manure, I did not consider the other parts of the equation. For example, the amount of manure excreted is not considered, so net GHG cannot be determined from these feeding regimes (Johnson et al., 2000). A literature review that looks at various studies examining each stage of the LCA could give better insight on this matter.

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