

**GREENHOUSE GAS EMISSIONS FROM GRASSLAND
PASTURE FERTILIZED WITH LIQUID HOG MANURE**

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

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©December, 2008

ABSTRACT

Trémorin, Denis G., M.Sc., University of Manitoba, October, 2008. Greenhouse Gas Emissions from Grassland Pasture Fertilized with Liquid Hog Manure. Major Professor; Mario Tenuta.

A study was conducted in 2004 and 2005 to determine the effect of liquid hog manure fertilization on greenhouse gas emissions from the surface of a grassland pasture in south-eastern Manitoba. The objectives of this research were to determine the effects of manure application, timing and soil moisture on greenhouse gas emissions from pasture soil, cattle dung and urine patches. Nitrous oxide (N₂O), methane (CH₄) and carbon dioxide (CO₂) emissions were determined from grassland soil surface, and from cattle dung and artificial urine patches. Liquid hog manure treatments were no manure (Control); 153 kg ha⁻¹ of available-nitrogen (N) (two year average) in spring (Spring); and 149 kg ha⁻¹ as half-rate applications in fall and spring (Split). Four field experiments were conducted on grassland plots. The static-vented chamber technique was used to estimate gas emission rates. Two of the experiments focused on the effects of manure application timing and soil moisture on greenhouse gas emissions from the grassland soil surface. The other two experiments focused on the effects of manure application and soil moisture on greenhouse gas emissions from cattle dung and artificial urine patches. Fresh cattle dung was collected from steers grazing adjacent pastures receiving the same three manure treatments. Artificial cattle urine treatments were generated by converting blood urea concentrations of the steers into urine-N concentrations.

Manure application increased ($P \leq 0.01$) cumulative N_2O emissions from the grassland soil surface with Control, Split and Spring treatments averaging 7, 43 and 120 $mg N_2O-N m^{-2}$, respectively. Of the two manure treatments, the Spring treatment emitted higher ($P \leq 0.10$) N_2O emissions than the Split treatment. Soil moisture was a major factor influencing the quantity and type of greenhouse gas emissions, with saturated areas emitting CH_4 during warm periods, whereas drier areas emitted N_2O . Nitrous oxide emissions from these dry areas were higher in manure-treated plots. Spring application increased root density by 45% in the top 5 cm of soil compared to the Control. An increase in soil organic carbon with root density may offset any increase in greenhouse gas emissions caused by manure treatment.

Cattle dung from Split and Spring treatments had higher cumulative N_2O emissions (30 and 82 $mg N_2O-N m^{-2}$, respectively) compared to dung from Control pastures (6 $mg N_2O-N m^{-2}$) over two study years. Dung from the Spring treatment emitted more N_2O ($P \leq 0.01$) than the other two treatments. All cattle dung patches emitted CH_4 after deposition though unaffected by manure treatment. Artificial urine having highest N concentration had greater ($P \leq 0.05$) cumulative N_2O emissions (690 $mg N_2O-N m^{-2}$) than urine with the lowest N concentration (170 $mg N_2O-N m^{-2}$). Drier soil locations emitted more N_2O from cattle dung and artificial urine patches than wetter areas.

This study demonstrated that Split application of liquid hog manure to grassland emitted less N_2O than a complete application in spring. Moisture greatly affected the location of N_2O and CH_4 emissions. Drier areas emitted more N_2O than wetter ones. Particularly, the findings indicate a need to assess grassland on periodically saturated soils as sources rather than sinks for CH_4 . Application of manure increased greenhouse

gas emissions from cattle dung and urine patches with urine potentially having the greatest impact because of their higher emissions of N_2O . An increase in root growth seems to offset greenhouse gas emissions from manure application.

ACKNOWLEDGMENTS

I thank Dr. Mario Tenuta for his guidance, encouragement, patience and friendship during the preparation of this Master's thesis. His enthusiasm for all things soil always made me realize that my research was important and that I could get this difficult task accomplished. I'm truly think that this project was made for me, and I feel that we worked well together on it, albeit a little slowly! A thank you also goes out to Dr. Don Flaten for his input, and for 'kicking me in the butt' on several occasions, and to Dr. Kim Ominski for her enthusiasm throughout this project and her input. I would also like to thank Dr. Wole Akinremi for serving on my committee and for providing assistance on several occasions.

This project would not have been possible without the support of government and industry groups. I would like to thank in particular the assistance of Heinz Reimer and Sheldon Stott of Hytek Ltd. in providing the land and assistance necessary to make this project work. I would also like to acknowledge the help of Dr. Graham Phipps and Tobin Harrison with Manitoba Water Stewardship for their assistance with rainfall and water table data.

I would like to thank Jordan Ramm, Emilie Novara, Steven Laurie, Amelia Hall and Tim Stem for field and lab assistance. Thank you to my project partners, Colleen Wilson and Joël Walkty for your assistance and encouragement. A thank you also goes to Dr. Gary Crow for his statistical expertise. A special thank you goes to Rob Ellis and Brad Sparling for technical assistance in the laboratory.

The support of family and friends has been invaluable during the course of this project, I don't think I can count how many times somebody asked me, "You're not done that yet? Get it done already!". That was a bigger motivator than anybody can imagine. Finally, I would like to thank Mom and Dad, who never asked about my ambitions to further my schooling. You have always supported me, and I have always felt that I could do anything that I wanted to do in life.

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ABBREVIATIONS

ADF	=	acid detergent fiber
ANOVA	=	analysis of variance
BUN	=	blood urea nitrogen
C	=	carbon
CH ₄	=	methane
cm	=	centimeter
CO ₂	=	carbon dioxide
CV	=	coefficient of variation
°C	=	degrees Celsius
DM	=	dry matter
DOY	=	day of year
EC	=	electrical conductivity
ECD	=	electron capture detector
Eq.	=	equivalent
FID	=	flame ionization detector
Fig.	=	figure
g	=	gram
g	=	gravity
GDD	=	growing degree-days
GHG	=	greenhouse gas
h	=	hour
Ha	=	hectare
HA	=	hippuric acid
H ₂ O	=	water
IPCC	=	Intergovernmental Panel on Climate Change
KCl	=	potassium chloride
kg	=	kilogram
km	=	kilometer
K ₂ SO ₄	=	potassium sulphate
L	=	liter
lbs	=	pounds
m	=	meter
M	=	molar
m ²	=	square meter
max.	=	maximum
mg	=	milligram
mL	=	milliliter
mm	=	millimeter
mS	=	millisiemens

mTorr	=	millitorr
mV	=	millivolts
N	=	nitrogen
N ₂	=	dinitrogen
N ₂ O	=	nitrous oxide
NaCl	=	sodium chloride
NDF	=	neutral detergent fiber
NH ₄ ⁺	=	ammonium
NH ₄ Cl	=	ammonium chloride
NO ₂ ⁻	=	nitrite
NO ₃ ⁻	=	nitrate
O ₂	=	oxygen
P	=	phosphorus
ppmv	=	parts per million by volume
PVC	=	poly-vinyl chloride
R ²	=	r-square
t	=	metric tonne
TCD	=	thermal conductivity detector
temp.	=	temperature
UTM	=	universal transverse mercator
UUN	=	urine urea nitrogen
μg	=	microgram
μL	=	microliter

1. INTRODUCTION

Increases in the atmospheric concentrations of greenhouse gases (carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O)) have caused an enhanced radiative forcing of the earth's atmosphere (climate change) (Intergovernmental Panel on Climate Change 2001). On a global basis, the emission of the 3 major greenhouse gases from agriculture account for about one-fifth of the annual increase in greenhouse gas concentrations in the atmosphere (Cole et al. 1997). Canadian agriculture accounts for about 10% of the total anthropogenic greenhouse gas emissions from all sectors (Desjardins et al. 2001). Globally, the agricultural sector produces 70% and 50% of nitrous oxide (N₂O) and methane (CH₄) emissions, respectively (Cole et al. 1997). In Canada, N₂O and CH₄ provide about 31 and 43%, respectively, of the total greenhouse gas emissions from the agricultural and agri-food sector (Desjardins et al. 2001).

Animal manures are important sources of N₂O, with an estimated 45% of N₂O from agriculture in Canada associated with the collection, storage and application of animal manure (Gregorich et al. 2005). Thus, there is significant potential to decrease N₂O emissions through improved manure management practices. The majority of CH₄ emissions from Canadian agriculture results from livestock production, in particular, enteric fermentation from ruminants (Gregorich et al. 2005). Most arable lands are actually sinks for atmospheric CH₄, as soil methanotrophs consume CH₄ as a source of energy and carbon. However, in localized areas with poor drainage, CH₄ production can occur on agricultural lands (Gregorich et al. 2005).

Unfertilized grass-based cattle pasture systems have typically low productivity. Many producers around the world fertilize these systems in order to enhance pasture productivity, and the productivity of ruminants grazing that land. If available, producers will use animal manures to fertilize these systems, as this source of nutrients is typically much less expensive than commercial fertilizer. Many studies have examined manure application to grassland and the resulting impact on emissions of N_2O and CH_4 from the soil. However, few studies have analyzed the impact of application timing, or the effects of a split-application, on greenhouse gas emissions. Even fewer studies have attempted a holistic approach to this system, examining the impact of manure application on greenhouse gas emissions from not only the soil, but also from the excreta of ruminants grazing manure-fertilized pasture. Manure application to grassland can have an impact on greenhouse gas emissions from excreta because fertilizing grasslands improves the quality of forage (protein content and organic matter digestibility) produced (Reid et al. 1966; Cohen et al. 2004). An increase in protein content has been shown to increase urea excretion from cattle (Archibeque et al. 2001; Basurto-Gutierrez et al. 2003) and may also impact N concentration in cattle dung. An increase in forage digestibility (due to increased vegetative growth in grasses) will increase decomposition rates of the dung produced by ruminants. An increase in N excretion from cattle urine and dung will increase N_2O emissions from both these patch types, and a change in dung decomposition rates may have an impact on CH_4 emissions from cattle dung. This study focused on the impact of manure application on N_2O and CH_4 fluxes from grassland soil and from cattle dung and urine.

The objectives of this thesis project were:

- 1) To study the impact of time of manure application on the greenhouse gas emissions (N_2O , CH_4 and CO_2) from a grassland soil, and to examine the control of soil moisture on the emission of these gases.
- 2) To determine if manure application to grassland pasture has an effect on greenhouse gas emissions from cattle dung and urine patches, and to examine the effect of manure application timing and soil moisture on greenhouse gas emissions from these patches.

This thesis consists of four chapters, the first being this introductory chapter which justifies the objectives of the thesis. The two following data chapters are in sandwich format; the first data chapter addresses greenhouse gas emissions from the grassland soil, and the second data chapter addresses greenhouse gas emissions from cattle dung and urine patches. The final chapter consists of a discussion tying together the two data chapters as well as placing the results into the context of whole system research.

1.1. References

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2. NITROUS OXIDE AND METHANE EMISSIONS FROM COARSE-TEXTURED GRASSLAND SOIL RECEIVING LIQUID HOG MANURE

2.1. Abstract

Trémorin, Denis G., M.Sc., University of Manitoba, August, 2007. Nitrous oxide and methane emissions from coarse-textured grassland soil receiving liquid hog manure. Major Professor: Dr. Mario Tenuta.

The objectives of this study were to determine the impact of timing of application of liquid hog manure and soil moisture conditions on the emission of the greenhouse gases, nitrous oxide (N₂O) and methane (CH₄) from a coarse-textured grassland soil. Liquid hog manure treatments were no manure (Control); 153 kg of available-nitrogen (N) (two year average) applied per hectare (ha) in the spring (Spring); and 149 kg applied per ha (two year average) as half-rate applications in the fall and spring (Split). Two field experiments were conducted. The first field experiment was conducted over a two-year period and examined the effect of time of manure application and soil conditions on temporal emissions of the gases (Plot Experiment). The second experiment examined the effect of soil moisture on the type and quantity of greenhouse gas emissions. Transects of sample positions along a gradient in soil moisture on a Control and Spring plot were used (Transect Experiment).

Manure application caused a significant increase ($P \leq 0.01$) in N₂O emissions from the grassland soil surface with Control, Split and Spring treatments producing average emissions of 6.7, 42.6 and 119.8 mg N₂O-N m⁻², respectively. Of the two manure treatments, the Spring treatment produced significantly higher ($P \leq 0.10$) N₂O emissions

than the Split treatment. The cumulative emissions of N₂O and CH₄ together as CO₂ equivalents were also significantly higher ($P \leq 0.01$) in manured than the Control treatment (9.8, 35.6 and 70.2 g CO₂ m⁻² for Control, Split and Spring treatments, respectively). Soil moisture content was a strong driver of the quantity and type of greenhouse gas emissions from grassland soil. The Transect Experiment confirmed that saturated soil conditions resulted in CH₄ emissions in the Control and to a slightly greater extent the Spring plot. In contrast, drier portions of the field produced greater emissions of N₂O for the Spring manure treatment compared to the Control plot examined. Slight topographical relief, coarse-texture and high water table at the site resulted in surprisingly differing patterns of emissions of the greenhouse gases.

This study showed that Split application of liquid hog manure to grassland produced less emissions of N₂O. Grasslands used for cattle production in Manitoba are often marginal lands for agriculture with high moisture conditions in portions of the fields. The findings indicate a need to assess these soils as sources rather than sinks for CH₄ in national greenhouse gas inventories.

2.2. Introduction

Managed pastures are highly productive compared to the forage productivity of rangeland pastures, as increased pasture productivity is the goal of the livestock producer (Bolan et al. 2004). In order to increase pasture productivity, many producers fertilize pastures with animal manures. This is especially true for grass-based pastures, as there are no legumes to replenish lost nitrogen from the system (Bolan et al. 2004). In the case of south-eastern Manitoba, there are beef cattle pasture systems that are fertilized with liquid hog manure from local hog barns. As in any soil system, the application of manure

to grassland has the potential of increasing soil nitrous oxide (N_2O) emission through enhanced denitrification and nitrification (Chadwick et al. 2000; Ellis et al. 1998). Additionally, ammonia-N in the manure has the potential of decreasing soil methane (CH_4) consumption, and the labile carbon in the manure may stimulate CH_4 production (Chadwick et al. 2000; Ellis et al. 1998; Lessard et al. 1997).

An important management goal to improve the sustainability of manure application to grassland will be to decrease N_2O emissions, as this is an important greenhouse gas emitted from soil and contributes to losses of nitrogen, an important plant nutrient. One of the more obvious N_2O abatement strategies would be to time manure application when it favours plant uptake of N (Chadwick 1997). In addition, the environmental factors which influence soil N_2O production (soil temperature, soil water content and crop growth) often differ during the year (Rochette et al. 2004). In Manitoba, Canada, it is prohibited for large farms (over 300 animal units) to apply animal manures during winter. Therefore, it is common practice to apply manure to grassland once during the growing season, either in the spring, summer or fall. There is a lack of knowledge in Canada regarding greenhouse gas emissions resulting from liquid hog manure application to grassland and even less is known about manure application to coarse-textured soil prone to both dry and wet conditions. In addition, the potential to decrease greenhouse gas emissions from manure-fertilized grassland by applying manure at two different times of the year (split application) has not been examined. The use of a split application with reduced N applied at each application has the potential to reduce N_2O emissions compared to a single application with larger quantities of N. This should reduce the rate of both nitrification and denitrification, and should reduce the possibility

of a large flush of ammonium (NH_4^+) and nitrate (NO_3^-) in soil. The application of manures in the fall may also reduce N_2O emissions, as soil conditions are cooler. These cool soil conditions reduce the rate of nitrification and denitrification in the soil while plant uptake can prevent nitrogen loss. Christensen (1983) has shown that variation in N_2O emission from manured grassland was influenced by temperature and that lower temperatures in the fall corresponded to lower N_2O fluxes.

Soil moisture is a very important determinant of the amount and type of greenhouse gas emitted from the soil. Increasing moisture can induce nitrification and then denitrification as the source of N_2O emissions. Further, very wet conditions can lead to anaerobic conditions suitable for methanogenesis and CH_4 emissions (Ambus 1998; Velthof et al. 1996; Velthof et al. 2000). Variation in soil moisture conditions is often related to topography, with lower slope positions generally having higher soil moisture contents than higher slope positions (Dunmola 2007). This can be due to collection of runoff in the lower slope positions, and the fact that water tables are closer to the soil surface in this same position.

Soil moisture content is the most important determinant of soil redox potential. Soil redox potential determines which processes, such as nitrification or denitrification, and methanogenesis or methanotrophy (methane consumption by soil bacteria) occur in the soil. Incomplete nitrification and denitrification produces N_2O from soil, and nitrification occurs where soil is drier and denitrification occurs where soil is wetter (redox potential $< +350$ mV). Methanogenesis only occurs when the soil is saturated (redox potential < -150 mV), and methanotrophy occurs under non-saturated soil conditions (Yu et al. 2001). In general, agricultural soils are considered to be sinks for

CH₄, however poorly drained portions of fields (ex. landscape depressions) are possibly sources of CH₄ at wet times of the year (Dunmola 2007). To date, coarse-textured soils with a high water table during snowmelt and after heavy rainfalls have not been examined for N₂O and CH₄ emissions. Since the type and quantity of greenhouse gas emissions is so dependent on soil moisture, it is important to clarify the relation of soil moisture in determining the pattern of greenhouse gas emissions from such soils.

The objective of this study was to determine N₂O and CH₄ emissions from coarse-textured grassland soil expressing both very low and very high moisture conditions and receiving liquid hog manure. Such lands are often used for forage production in livestock systems. In 2004 and 2005, application of one-half rates of manure in spring and fall and the resulting gas emissions were compared to a full rate of manure in spring. Soil moisture was suspected to be the major determinant of greenhouse gas emissions with local high water table conditions resulting in methane production rather than consumption. Greenhouse gas emissions from liquid hog manure application along a transect of wet to dry moisture gradient in the field was thus examined for three dates during 2005.

2.3. Materials and Methods

2.3.1. Site Location and Description

The site comprised of 39 hectares in the Rural Municipality of La Broquerie, (Manitoba Land Survey SE 20-5-8E, UTM 14U 683348 5475081) located 85 km southeast of Winnipeg, Manitoba. The site is located within the Interlake Plain of the Boreal Plains Ecoregion in South-eastern Manitoba. The ecoclimate of this region is

classified as subhumid low boreal and the native vegetation of the ecoregion is dominantly deciduous boreal forest (National Ecological Framework for Canada, Environment Canada). The town of Steinbach, Manitoba, located approximately 18 km northwest of the study site, has a mean annual temperature of 2.7°C, with average January and July temperatures of -17.4°C and 19.1°C, respectively (Weather Records Office, Environment Canada, 1975-2005 average). Average total precipitation at Steinbach is 539 mm, with a mean annual rainfall of 440 mm (1975-2005 average).

The Canadian Agricultural Capability Class of the site is Class 3m, which is marginal for annual crop production due to the potential for a lack of soil moisture caused by rapid drainage and low moisture holding capacity of the coarse-texture soil at the site. According to a detailed soil survey report, the soil series that are present at the site are Berlo loamy fine sand (70%) and Kergwenan loamy sand to gravel (30%) (Canada-Manitoba Soil Survey Report D49, 1953). The Berlo soil series is an imperfectly drained Gleyed Dark Gray Luvisol developed on strongly calcareous deltaic sediments, while the Kergwenan soil series is an imperfectly drained Dark Gray Chernozem developed on strongly calcareous sandy and gravelly outwash (personal communication, Peter Haluschak, Director of Soil Survey, Manitoba Agriculture, Food and Rural Initiatives). The topography of the site is very gently sloping to level and soil water permeability is rapid. Internal drainage is imperfect due to a rapidly fluctuating water table that may rise to the soil surface in some areas during snowmelt and after periods of prolonged and heavy rainfalls. The eastern side of the research site has coarse-textured surface materials that extend from the ground surface to greater than 7.5 m, and from the ground surface to 1.5 to 2.1 m on the western side. The western half of the research site is underlain by

impervious clay that is 3 to 4.5 m thick (personal communication, Dr. Graham Phipps, Manitoba Water Stewardship). This clay layer creates a perched water table in the spring and after periods of heavy rainfall, restricting water drainage, especially at the far western edge of the site. The presence of this clay layer was confirmed by a ground electromagnetic survey of the site conducted at the end of the summer in 2003 (Fig. 2.1). The vegetation of the site consists of predominantly grasses (*Agropyron repens*, *Poa pretensis*, *Phleum pretense*, *Dactylis glomerata*), with some legumes (*Medicago sativa*, *Lotus corniculatus*, *Trifolium spp.*) and forbs. Some of these species were established as a result of seeding during the spring of 2000, others were not introduced at seeding (*Agropyron repens*) and became established as part of a natural succession. The site had not been fertilized since seeding in 2000.

The experimental design for this project was a factorial with three treatments of manure and two forage utilization strategies (Figures 2.2. and 2.3.). Each treatment was replicated twice. The boundaries of the two replicates were delineated according to the electromagnetic survey of the site with the wetter western section being Replicate 1 underlain by clay. The manure treatments were: no application (Control); and two liquid hog manure treatments; a target application rate of 125 kg of plant available N per hectare (ha) applied in the spring (Spring); and half-rates (62.5 kg of plant available N per ha) applied as two applications, one in the fall, the other in spring (Split). The forage utilization treatments were cattle grazing and mechanical haying.

The size of the grazed plots varied according to the area expected to support 10 grazing steers, with manure-treated plots being smaller as the productivity of these plots was expected to be higher. The hayed plots were about 1.2 ha. The two control cattle

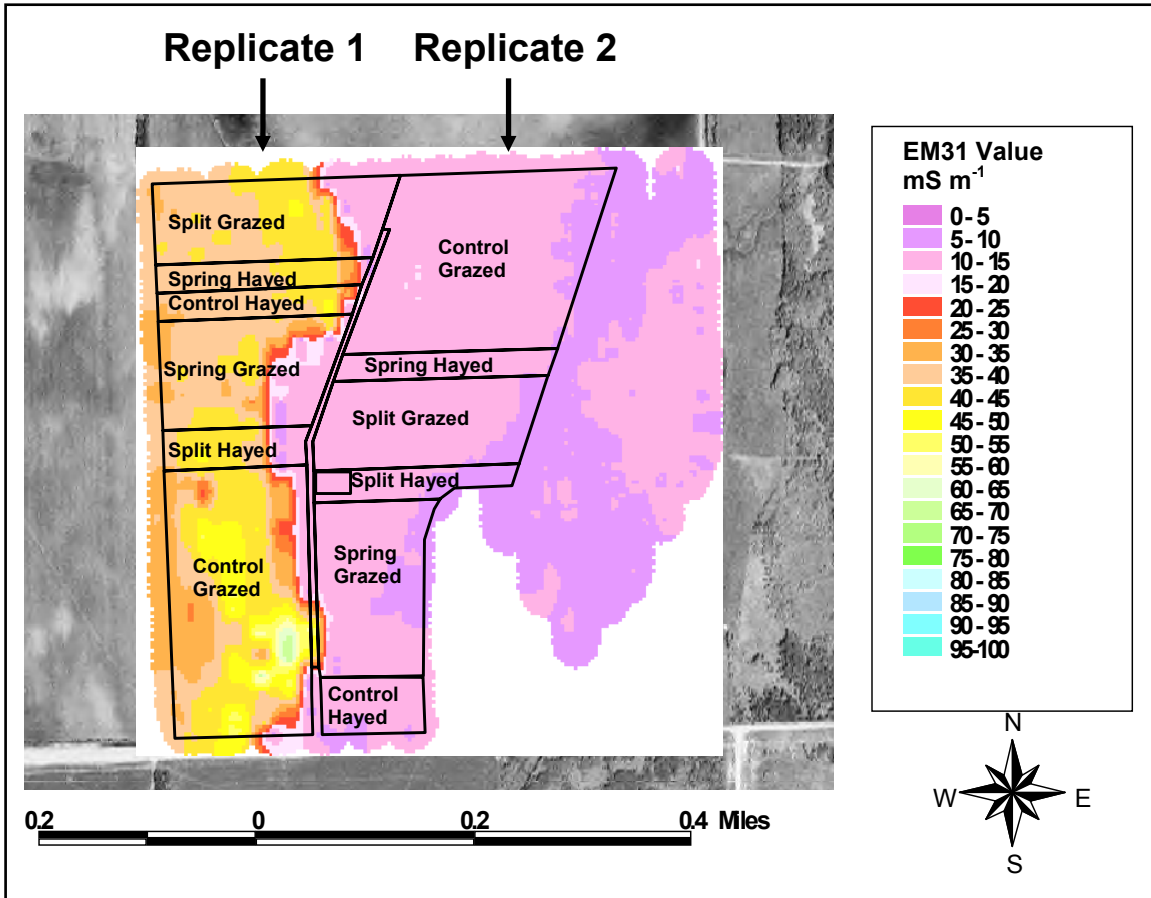


Figure 2.1. Results of a ground electromagnetic (EM31, Geonics Ltd.) survey of the La Broquerie Pasture Project Research Site conducted by the Prairie Farm Rehabilitation Administration in late summer, 2003. The western section of the site shows to be underlain by clay (readings 15-60 mS m⁻¹) and the eastern section by coarse material (readings 0-15 mS m⁻¹).

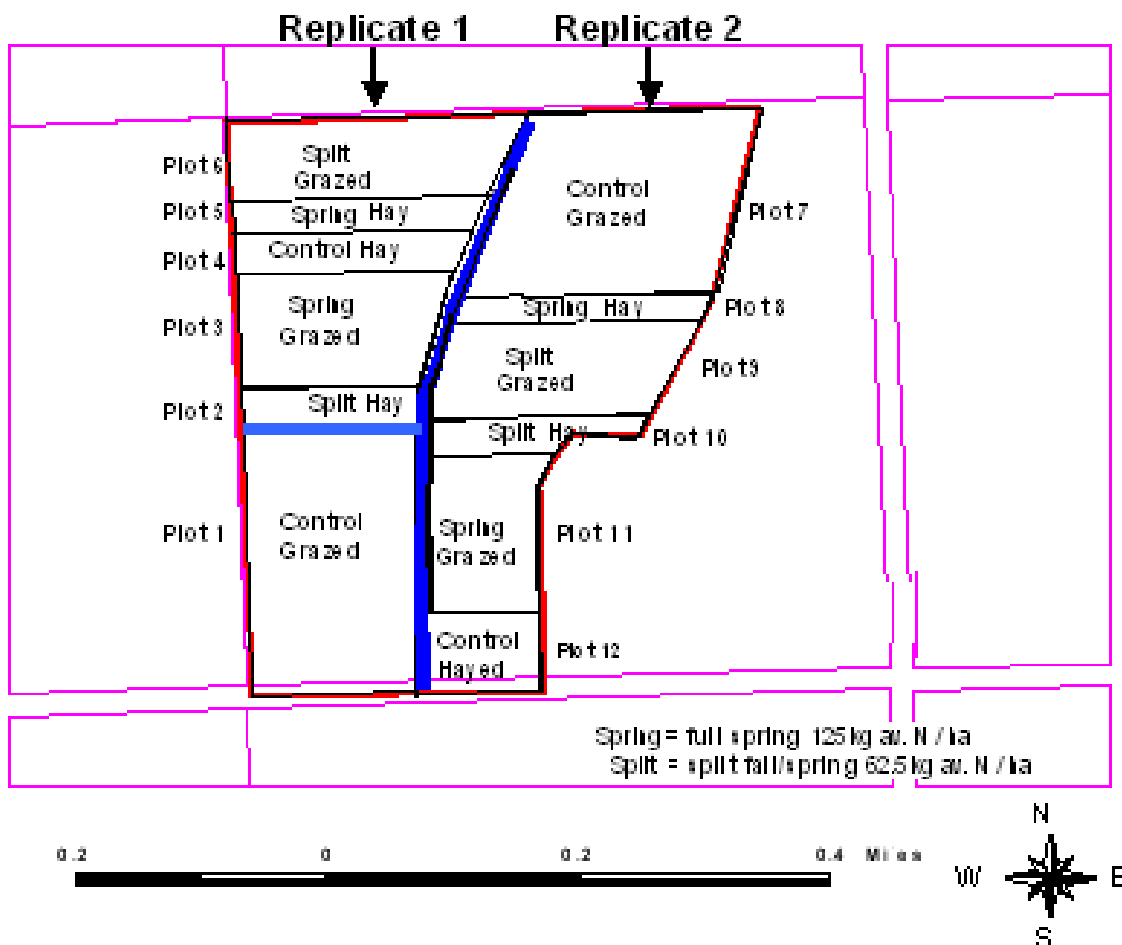


Figure 2.2. Plot layout and treatments at the La Broquerie Pasture Project Research Site.



Figure 2.3. Aerial photo of the La Broquerie Pasture Project Research Site taken July 2004. Photo faces north, alley separating replicates can be seen up the middle of the site with Replicate 1 plots to the left and Replicate 2 plots to the right (photo credit: Gary Martens, Department of Plant Science, University of Manitoba). Distinguishable as a result of differing fertility are unmanured Control plots of pale green compared to Split and Spring manure treatments being darker green.

pastures were 8 ha, the four manured cattle pastures were 4 ha. Cattle were grazed using a put and take system in which, the number of cattle in the pasture plots varied with forage availability (steers added or removed depending on forage growth and availability). The project began in the fall of 2003 with baseline soil sampling for nutrients and soil properties, followed by manure application to the Split treatments that same fall.

2.3.2. Manure Application

Liquid hog manure was obtained from four finishing barns immediately south-east of the site. These barns, operated by Hytek Ltd., supply manure to a primary, secondary and tertiary earthen lagoon system. Manure was obtained from the primary cell after a minimum of 3 hours of agitation to mix solids at the bottom of the cell as well as possible. Agitation continued throughout the application process. Manure application rates with a target of 125 kg of plant available N / ha were used as recommended by Manitoba Agriculture, Food and Rural Initiatives. Available N was calculated as the sum of available ammoniac-N (manure ammonical-N less 25% loss of volatilization for surface application of manure to cover crops in cool conditions) and available organic N in the manure (assume 25% of organic N available during the cropping year of application) (Tri-Provincial Manure Application and Use Guidelines, Manitoba Version, 2004). Before manure was applied to the field, a sample was taken and ammonical-N was estimated using a Novameter© (Agros, Lidköping, Sweden). Organic N was determined using a hydrometer to measure the percentage of dry matter in the liquid hog manure. Organic N was calculated as 3.33% of dry matter in the liquid hog manure as reported for liquid hog manure in Manitoba (Fitzgerald and Racz 2001). Manure samples

were also taken at the start, middle, and end of each day of application, and samples were sent to a commercial laboratory for analysis (Norwest Laboratories, Winnipeg, Manitoba). Manure was analyzed for nitrogen ($\text{NH}_4\text{-N}$ and organic N) and phosphorus (Tables 2.1 & 2.2). For the most part, there was little discrepancy between the field test for ammonical-N and solid content compared to the results from the commercial laboratory. However, due to a faulty gauge of the field test unit, the amount of available N was overapplied in spring and fall of 2004. This problem gauge was replaced after the fall of 2004. In order to prevent future use of a faulty gauge, a solution of NH_4Cl (eq. 30 lbs of $\text{NH}_4\text{-N}$ / 1000 US gallons) was freshly prepared before use to calibrate the gauge of the field test unit.

Liquid hog manure was surface-applied to all plots. In the fall of 2003 (October 21, Day of year (DOY) 294), hog manure was applied using a drag-line system supplying a boom of six splash-plates about 1 m above the soil surface. All following manure applications used a tractor-driven tanker system (27,350 L capacity) with a single, high-trajectory splash-plate. In order to adjust the rate of application of the equipment, we set a tractor-PTO speed and adjusted the ground speed. During the spring of 2004, all treated plots in replicate one, except the grazed Spring treatment, received manure on May 10 (DOY 131), but application had to be stopped because of a storm on May 11 (DOY 132) during which 16 cm of snow and 24.4 mm of rain fell. Application continued on May 25 (DOY 146) once the snow had melted. Table 2.3 shows the negligible difference in applied nutrients over these two application dates during the spring of 2004. Also of note, the splash-plate used during the spring of 2004 did not provide good

Table 2.1. Manure application rates and nutrients applied per hectare.

Treatment & Time of Year	Date of Application	Rate L ha⁻¹	Moisture %	Available N kg N ha⁻¹	Total N kg N ha⁻¹	Ammonia kg N ha⁻¹	Organic N kg N ha⁻¹	Phosphorus kg P ha⁻¹
Split fall 03	21-Oct-03	25700	96.4	68	111	82	30.1	17.7
Split spring 04	May, 2004	26947	93.7	83	152	91	61.4	35.7
Total Split 03/04		52647	95.0	151	263	173	91.5	53.4
Spring 04	May, 2004	53894	93.7	166	303	181	122.8	71.3
Split fall 04	24-Oct-04	36019	98.1	77	116	95	19.9	6.0
Split spring 05	26-Apr-05	16043	87.9	70	130	74	56.1	30.9
Total Split 04/05		52062	93.0	147	246	169	76.0	36.9
Spring 05	26-Apr-05	32085	87.9	139	260	148	112.2	61.8
Split fall 05	13-Oct-05	17080	93.7	66	119	81	35.2	18.2
Average Total Split¹		52354	94.0	149	263	171	83.8	54.0
Average Spring²		42990	90.8	153	282	165	117.5	69.7

¹ Average of Split treatment (including both fall and spring applications) from 2004 and 2005 study years.

² Average of Spring treatment from 2004 and 2005 study years

Table 2.2. Physical characteristics and nutrient composition of manure sampled during manure application activities from the fall of 2003 to the spring of 2005.

Manure composition	Spring (n=12)		Split (n=19)	
	Mean	CV (%)	Mean	CV (%)
moisture content (%)	9.4	31	7.0	56
pH	6.9	3	6.9	3
EC mS m ⁻¹	20.6	10	20.3	9
total N (%)	0.61	8	0.53	23
NH ₄ -N (mg N L ⁻¹)	3564	5	3341	11
P (mg P L ⁻¹)	1508	13	1136	49
total N applied (kg ha ⁻¹)	282	8	263	12
total P applied (kg ha ⁻¹)	70	8	54	43

Table 2.3. Comparison of two manure application dates during the spring of 2004: manure application rates and nutrients applied per hectare.

Date of Application	Rate L ha⁻¹	Moisture %	Available N kg N ha⁻¹	Total N kg N ha⁻¹	Ammonia kg N ha⁻¹	Organic N kg N ha⁻¹	Phosphorus kg P ha⁻¹
10-May-04	53893	93.4	170	304	189	116	73
25-May-04	53893	93.9	161	302	173	129	70

Note: Manure was applied to both Split and Spring treatment plots, but only Spring application rate is shown for comparison purposes.

distribution of manure. There was evidence of this during the 2004 growing season, as forage growth increased in strips along the path of the manure applicator. A new splash-plate was installed for the fall 2004 manure application (October 24, DOY 298) that did not result in uneven forage growth.

2.3.3. Field Studies

2.3.3.1. Plot Study (2004-2005). A plot study was conducted to compare the greenhouse gas emissions from the different treatments at the field site. This study was conducted on the hayed plots in order to eliminate disturbance from cattle (urination, defecation and physical disturbance of study areas by cattle) and was carried out during the 2004 and 2005 growing seasons. The static-vented chamber method was used to measure greenhouse gas emissions from the soil throughout the year (Hutchinson and Livingston 2002). The chambers were positioned during the spring of 2004 by separating the hayed plots into six equal sections from west to east, and placing one chamber within each section, for a total of six chambers per plot. Chambers were placed in each section by tossing the collars into a section while standing at the edge. The chamber was placed where the collar settled. This method of placing chambers was used in order to encompass as much of the soil moisture variability in a plot as possible as observation indicated a moisture gradient from west to east in each of the plots. The number of chambers placed in each plot was limited to six as this was the maximum number of chambers that could be sampled during a 45 minute period by one individual allowing for the collection of four gas samples per chamber at 15 minute intervals. In 2004, greenhouse gas sampling and soil sampling began on May 17 (DOY 138) for replicate 1, and May 25 (DOY 146) for replicate 2, and the last day of sampling was on Nov 16

(DOY 321). Start dates differed due to the snowfall that interrupted manure application in the spring of 2004. In 2004, sampling did not occur from June 28 (DOY 180) to July 19 (DOY 201), and from September 23 (DOY 267) to October 26 (DOY 300) to harvest hay in the plots. In 2005, sampling began on April 7 (DOY 97) and continued to November 5 (DOY 309). Sampling was stopped once for haying from July 13 (DOY 195) to July 28 (DOY 209).

2.3.3.2. Gas Flux Measurement and Analysis. The static-vented chambers used consisted of a collar with a removable lid which were inserted into the soil. The collar was made from 20.3 cm interior diameter (i.d.) white PVC pipe (schedule 40) cut into 10 cm lengths; the bottom 2 cm beveled to an edge to allow easier insertion into soil. The lid consisted of a 0.6 cm thick grey PVC disk with a diameter of 23 cm. The lid was fitted with a Nalgene tube (0.32 cm i.d., 10 cm length) to allow equilibration of chamber pressure to atmospheric pressure but to also minimize gas diffusion loss. A rubber septum (Sub-Seal, Sigma Aldrich) was also fitted to the lid to allow insertion of a syringe needle to collect gas samples. Both the collar and lid were fitted with rubber gaskets fashioned from tire inner tubes (Canadian Tire Corporation). The rubber gaskets on the surface of the chambers were painted white and the top of the lids were covered with aluminum foil to minimize solar heating. Four screws were inserted into the sides of the collar and elastics were stretched from one screw across to another to seal the lid to the collar.

Collars were inserted into the soil using a knife and rubber mallet to a depth of 3 cm into the soil and left in place until forage harvesting required their removal. Gas sampling began by compressing the lid to the collar edge using elastic bands, and then

gas samples taken from the headspace of the chamber at 15, 30 and 45 minutes following closure of the chamber. At each interval, 20 mL gas samples were taken through the septum on the chamber lid using a 20 mL syringe (Becton-Dickinson) with a 23-gauge luer-lock needle. The sampled gas was then injected into 12 mL Exetainer glass vials (Labco, UK). Prior to their use, the Exetainer vials were sealed with silicone and were flushed with helium three times and evacuated to < 350 mTorr. For each plot, three 20 mL samples of atmospheric air were taken at ground level before placing the lids on the collars, these samples represent the headspace at the start of the measurement. As a check of the vial handling and gas storage procedures, two vials of 20 mL samples of two standard gas mixtures (N₂O, CH₄ and CO₂; BOC Edwards) were put into evacuated Exetainers (Labco Int., Houston, TX) and handled in the same way as the other gas samples on every day of gas sampling. Gas samples were collected in the morning and completed by noon to prevent measurements being taken during the hottest period of the day. Forage within the collars was clipped to 3 cm before emission determinations to simulate grazing, and to allow placement of lids on the collars.

After sampling, Exetainers were returned to the lab, sealed with silicone sealant, and gases were analyzed for CO₂, CH₄ and N₂O using a Varian CP-3800 gas chromatograph (Varian Canada) fitted with electron capture (ECD), flame ionization (FID), and thermal conductivity (TCD) detectors and with a Combi-PAL autosampler (CTC Analytics). The gas chromatograph was regularly calibrated using laboratory prepared dilutions of pure gases. The gas concentration of the two commercially prepared reference standards (high and low concentrations) were determined by the Soil Ecology Laboratory and these included every 15 samples for each run of the gas chromatograph.

In 2004, the Low standard consisted of 193 $\mu\text{L/L}$ CO_2 , 4.2 $\mu\text{L/L}$ CH_4 and 0.421 $\mu\text{L/L}$ N_2O , while the High standard consisted of 921 $\mu\text{L/L}$ CO_2 , 8.6 $\mu\text{L/L}$ CH_4 and 1.100 $\mu\text{L/L}$ N_2O . In 2005, the Low standard contained 177 $\mu\text{L/L}$ CO_2 , 4.9 $\mu\text{L/L}$ CH_4 and 0.299 $\mu\text{L/L}$ N_2O , while the High standard contained 1000 $\mu\text{L/L}$ CO_2 , 10.5 $\mu\text{L/L}$ CH_4 and 0.960 $\mu\text{L/L}$ N_2O . If the reference standard was off by more than 5% of true value, the sample analyses were repeated or the gas chromatograph columns rejuvenated by high temperature heating. Storage time did not affect the gas concentrations of the reference standards used for this experiment (typical time 1 to 5 months).

In order to calculate greenhouse gas emissions, the following steps were followed. Gas concentrations were determined by gas chromatography as ppmv ($\mu\text{L gas} / \text{L total gas}$). This concentration was converted to the mass of gas in the headspace of the chamber using the ideal gas law, the known molar mass of the gas and air temperature at time of sampling (measured during sampling with digital thermometer). The mass of gas in the chamber was then expressed on a chamber area in square meters basis. Finally, gas emission rate was determined from the slope of the linear regression plot of gas accumulated over the sampling periods (0, 15, 30, 45 minute sampling times). R-square (r^2) values of the linear regression plots were calculated for each chamber at each sampling date. If the r^2 value was lower than 0.9, the gas concentrations for each sampling time were examined, and if one gas sample was the cause of this deviation, this sample was taken out of the calculation of the slope. The final gas emission value was given as $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ for N_2O , $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ for CH_4 , and $\text{mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ for CO_2 .

2.3.3.3. Soil Sampling and Analysis. Soil samples to a depth of 5 cm were taken to determine factors driving gas emissions; NH_4^+ , NO_3^- and gravimetric moisture content. Soil sampling occurred on a weekly basis until the end of June for both years of the study; sampling was more irregular after this time due to haying and manure application activities on the plots. Five soil samples were taken with an Oakfield tube sampler (diameter = 1.9 cm) from a 2 m radius area around a collar position. The soil samples were placed into one polyethylene bag, stored in a picnic cooler with an ice pack until their return to the laboratory, where they were placed at -20°C . Soil samples were then thawed and gravimetric moisture contents determined by drying 10-g sub-samples at 105°C for 24 hours. The rest of the soil samples were then air-dried, ground with a mortar and pestle, and passed through a 2-mm mesh screen in order to remove gravel and coarse organic material. The ground and sieved samples were extracted in a 1:5 soil/solution ratio with 0.5M K_2SO_4 solution. Twenty-five mL of K_2SO_4 solution was added to 5 g of soil in 50 mL conical polypropylene tubes (Fisherbrand) and agitated for 30 minutes at 120 oscillations per minute on a reciprocating shaker. The mixture was then centrifuged at $1350 \times g$ for 90 seconds; then 15 mL of the clear supernatant removed and placed into scintillation vials, and frozen until analysis. These solutions were then analyzed colorimetrically for NH_4^+ , NO_3^- and nitrite (NO_2^-) using a Technicon II Autoanalyzer (Technicon Instrument Corporation). Ammonium was analyzed using the Phenate Method, while nitrate and nitrite were analyzed using the Azo-Dye Method with cadmium reduction for the detection of nitrate.

2.3.3.4. Transect Study (2005). Results of the plot study indicated a relationship between soil moisture and greenhouse gas emissions. A transect study was conducted to

determine how a gradient in soil moisture controls both the types (N_2O and CH_4) and amounts of greenhouse gases emitted from non-treated and manure-treated grassland. The Control and Spring hayed plot of Replicate 1 were chosen for this experiment because these plots are adjacent to each other and from observation had strong moisture gradients, with wetter conditions at the west end of the plots. The static-vented chamber method was used for gas emission determination as described previously.

Two parallel transects of 30 chambers each were placed in the Control and Spring hayed plots of replicate 1. The 30 chambers were placed 9 m from each other. In order to avoid any border effects along the edge of the plots, the parallel transects were placed 8.8 m apart, with each transect being 4.4 m from the adjacent plot. Greenhouse gas emissions were sampled on three days (June 2, 6, and 13, 2005) (DOY 153,157,164).

For each sampling date, soil sampling and analysis were also conducted as described previously. Soil samples were taken June 2, 13 and 24 (DOY 153,164,175) to determine gravimetric moisture content, plant available ammonium (NH_4^+) and nitrate (NO_3^-). Volumetric moisture content (0-6 cm) determinations were attempted in the field using a DeltaT™ Theta Probe (ML2X; Delta-T Devices, Cambridge, UK). However, great spatial variation in soil bulk density because of gravel and high concentration of roots prevented reliable calibration of the sensor. In addition, water would drain or be pushed out of soil samples collected from saturated areas at the western end of the transects, reducing the ability to calibrate the sensor. As a result, gravimetric moisture content is solely reported.

2.3.4. Rainfall, Air Temperature, Soil Temperature and Water Table

Rainfall was monitored in 2004 and 2005 at the southeast edge of the site with a tipping bucket rain gauge (Rain 110, Madgetech). Air temperature data for both years was obtained from the Steinbach airport located 11.7 km to the north-west. Soil temperature beside each chamber and ambient air temperature were measured at each greenhouse gas sampling event. A Traceable Longstem Thermometer (Fisher Scientific Company, Nepean, ON) was used to record these temperatures. Soil temperature was measured at a depth of 2.5 cm and ambient air temperature was taken by holding the thermometer 60 cm above the soil surface in the shade. Manitoba Water Stewardship collected daily water table measurements using a Telog WLS-31 data logger with pressure transducer (Telog Instruments Inc., Victor, NY, USA) inside a water monitoring well located at the NE edge of the site.

2.3.5. Data Analyses

All statistical analyses were performed using SAS version 8 software (SAS Institute Inc. 2000).

2.3.5.1. Plot Study. Two tests were performed to determine manure treatment differences of gas emission in the plot study, a repeated measures test and a general ANOVA on the cumulative GHG emissions from the plots. Correlations and simple regression analysis were also performed, to observe relationships between gas emissions and measured soil parameters. For both tests, the two replicates and two years of the study were combined to create four replicate-years, which was considered a random effect in the models tested.

2.3.5.1.1. Repeated Measures Test. A repeated measures test was performed to determine the time period when there were treatment differences due to manure application. The data analyzed for the repeated measures test included the N₂O and CH₄ emission data as well as the combined CO₂ equivalents of these two gases. The combined CO₂ equivalents were calculated by applying Global Warming Potential factors (100 year mean) of 21 and 310 for mg of N₂O and CH₄, respectively. Before the analysis could be performed, data gaps in between gas sampling days had to be filled by linear interpolation (Pennock et al. 2006). The data analyzed by this test included eight consecutive periods of 200 growing-degree-days (GDD), with the periods beginning after spring manure application. The data was separated into periods of GDD instead of days to address different application dates of manure and weather conditions between years. Growing-degree days should be used in this case as soil microbial activity is strongly driven by temperature.

The formula used to calculate GDD was the following:

$$GDD = \left(\frac{\text{Maximum_temperature} + \text{Minimum_temperature}}{2} \right) - \text{Base_temperature}(0)$$

The base temperature for the calculation of the GDD periods was 0°C.

These eight periods encompassed the time in which the majority of gas emissions occurred in both years. For Replicate 1 in 2004, the GDD periods began on May 17 (DOY 138), while the GDD periods for Replicate 2 began on May 26 (DOY 147) because of a delay in spring manure application. In 2004, the GDD periods ended on September 3 and 8 (DOY 247, 252) for Replicates 1 and 2, respectively. In 2005, the GDD periods encompassed the time between April 28 (DOY 118) and August 2 (DOY

214), much shorter than 2004 because of the warmer temperatures in 2005. The GDD periods encompassed approximately one to two weeks, depending on daily maximum and minimum air temperature of the particular GDD period. Gas emissions measured after fall manure application was not included in this analysis. It is important to limit the numbers of periods in a repeated measures test, as the strength of the analysis decreases with the number of periods included. Including all the data from this study would have decreased the strength of the analysis and would have decreased the possibility of finding significant differences between treatments.

A summation of data from each period was calculated and this data was analyzed using the repeated measures test after a $\log(x+y)$ transformation of the data was made to improve the normal distribution of the data set. Manure treatment, GDD period and the treatment*period interaction were specified as the fixed effects in the model statement. Replicate-year, the treatment*replicate-year interaction, and the treatment*replicate-year*period interaction were specified as random effects. PROC MIXED (SAS Institute Inc. 2000) was used for the analysis of variance of N₂O, CH₄ and the combined CO₂ equivalents of these two gases because of non-homogeneity of variance across manure treatments. Analysis with PROC MIXED was performed in two ways; one by analyzing the data assuming equal variance across all treatments, and the other by analyzing the data allowing for unequal variance in all treatments. The results from the latter analysis were determined to be better than the first if the Fit Statistic associated with the second analysis was lower than the Fit Statistic of the first analysis, and the difference was larger than the chi-square statistic associated with the degrees of freedom of the test. LSMEANS were then calculated to test for significant differences between the treatments

over the eight GDD periods. In periods where significant treatment effects were observed, Fisher's Protected LSD test determined minimum significant differences between treatments at $\alpha = 0.05$.

2.3.5.1.2. Cumulative Gas Emissions. A general ANOVA was used to determine if there were significant effects of manure treatment on the cumulative emissions of N₂O and CH₄, as well as the combined greenhouse warming potential (CO₂ equivalents) of these two gases. Again, linear interpolation was used to fill in gaps in data where sampling did not occur (Pennock et al. 2006). Just as in the repeated measures test, the cumulative gas emissions had to be log transformed to improve the normal distribution of the data set. For this test, manure treatment was specified as the fixed effect, while replicate-year and the treatment*replicate-year interaction were considered as the random effects of the model. Both PROC GLM (SAS Institute Inc. 2000) and PROC MIXED were used to perform ANOVA on the data set, depending if the variances of the standard deviations were distributed homogeneously or not across the treatments, respectively. LSMEANS were then calculated to test for significant differences between the treatments. Where significant treatment effects were observed, Tukey's test determined the minimum significant difference between manure treatments at $\alpha = 0.05$.

2.3.5.1.3. Correlations and Scatterplots. PROC CORR (SAS Institute Inc. 2000) was used to calculate Spearman correlation coefficients between the several dependent (N₂O, CH₄ and equivalent CO₂ emissions) and independent (soil NH₄⁺, soil NO₃⁻, soil temperature and soil moisture) variables measured. Correlation analysis was only performed for data in which both soil and gas samples were collected on the same day. Scatterplot graphs were also created between variables if there were strong correlations.

Data collected in early spring before manure application in 2005 was not used for these analyses, as data was not collected in 2004 during this period.

2.3.5.2. Transect Study. Scatterplots were created for the June 2 and the June 13 data, as these were the days when both gas and soil samples were collected. These figures were used to demonstrate obvious relationships between greenhouse gas emission and soil moisture.

2.4. Results

2.4.1. Manure Application

Throughout this study, dry matter content and the concentration of total N and total phosphorus (P) of manure varied a great deal for fall and spring manure applications. Analysis of manure samples at the start, middle and end of each application day showed little variation in dry matter content, and N and P concentrations (data not shown). The lack of variation seen throughout each pumping activity is due to the relatively small portion of manure removed from the primary manure cell (approximately 1,060,000 of the total 6,771,000 L) may explain the consistency of the manure being pumped. The primary cell used was always at capacity at the start of pumping. The manure was thoroughly mixed for 3 hours using an agitator prior to commencement of pumping. Mixing of manure throughout the application process was insured by continued agitation between pumping of manure to the application tank.

Applying manure to the target rate of 125 kg of available N per ha was difficult due to the problems with in-field measurements. The gauge on the Novameter malfunctioned during the fall 2004 application without our knowledge, and the use of a

check solution for the 2005 applications insured good working order of the Novameter. The Novameter as a tool to determine the NH_4^+ in liquid manure is nevertheless limited, as it underestimates the concentration of NH_4^+ in manure. This is especially true in manures with a high concentration of dry matter, which as was used for this experiment (personal communication, Sheldon Stott, Hytek Ltd.). The average application rate (2004 and 2005) to the Spring treatment plots was 42,990 L per ha, and 52,354 L per ha was applied to the Split treatment plots (fall and spring applications combined) as indicated in Table 2.1. The average available N per hectare applied to the Spring treatment plots was 153 kg of N, while an average of 149 kg of N was applied to the Split treatment plots. The average total N applied to the Spring treatment plots was 282 kg N per ha, and 263 kg N per ha was applied to the Split treatment plots. The majority of this N was in the form of NH_4^+ , an average of 165 kg of NH_4^+ -N per ha was applied to the Spring treatment plots; the Split treatment plots received an average of 171 kg of NH_4^+ -N per ha. The average rate of total P application to the Spring treatment plots was 69.7 kg P per ha, while the Split treatment plots received 54.0 kg P per ha (Table 2.1). Table 2.2 is included in order to show the small difference in nutrient application rates between the two different application dates during the spring of 2004. The P content of the manure was more variable than N content between applications and was lower in the fall than in the spring and resulted in the lower P application rate to the Split treatment plots (Tables 2.1 and 2.3). This is because the manure storage is emptied during summer so that there is less dry matter accumulated in the first cell in fall than in spring. Increased amounts of P applied to the plots during certain applications (springs of 2004 and 2005) correspond to higher concentrations of P and dry matter content in the liquid hog manure.

2.4.2. Plot Study

2.4.2.1. Gas Emissions in 2004 and 2005. Generally, soil emissions of N₂O, CH₄ and respired-CO₂ gas increased after manure application. There were definite patterns of N₂O and CO₂ emissions over time that were consistent between years. Each gas will be discussed in the following sections.

2.4.2.1.1. Nitrous Oxide. Soil N₂O emissions from the plots were generally highest from the Spring treatment plots, followed by the Split treatment plots and then finally by the Control plots (Fig. 2.4). The Control plots emitted very little N₂O, and the maximum measured emission from a sample position in the manured plots was over 15 times larger than that from the maximum from a sample position in the Control. Nitrous oxide emissions generally began shortly after spring manure application (Fig. 2.4). In 2004, N₂O emissions from plots fertilized with liquid hog manure dropped off by July 29 (DOY 211). In 2005, plots fertilized with liquid hog manure had N₂O emissions that generally lasted longer than in 2004 with emissions from the Split treatment plot on Replicate 2 dropping by August 15 (DOY 227). This longer period of emissions occurred despite the fact that manure was applied earlier to the plots in 2005 than in 2004. After fall manure application in 2004 and 2005, the Split treatment plot in Replicate 2 produced some N₂O emissions (average of 25.7 μg N₂O-N m⁻² h⁻¹) whereas other plots did not. This same plot also produced emissions of N₂O in the early spring of 2005 (average of 52.1 μg N₂O-N m⁻² h⁻¹), before spring manure application.

When N₂O emissions were occurring from the soil, there was high variability both between replicates and within plots. Generally, plots that were drier produced the highest

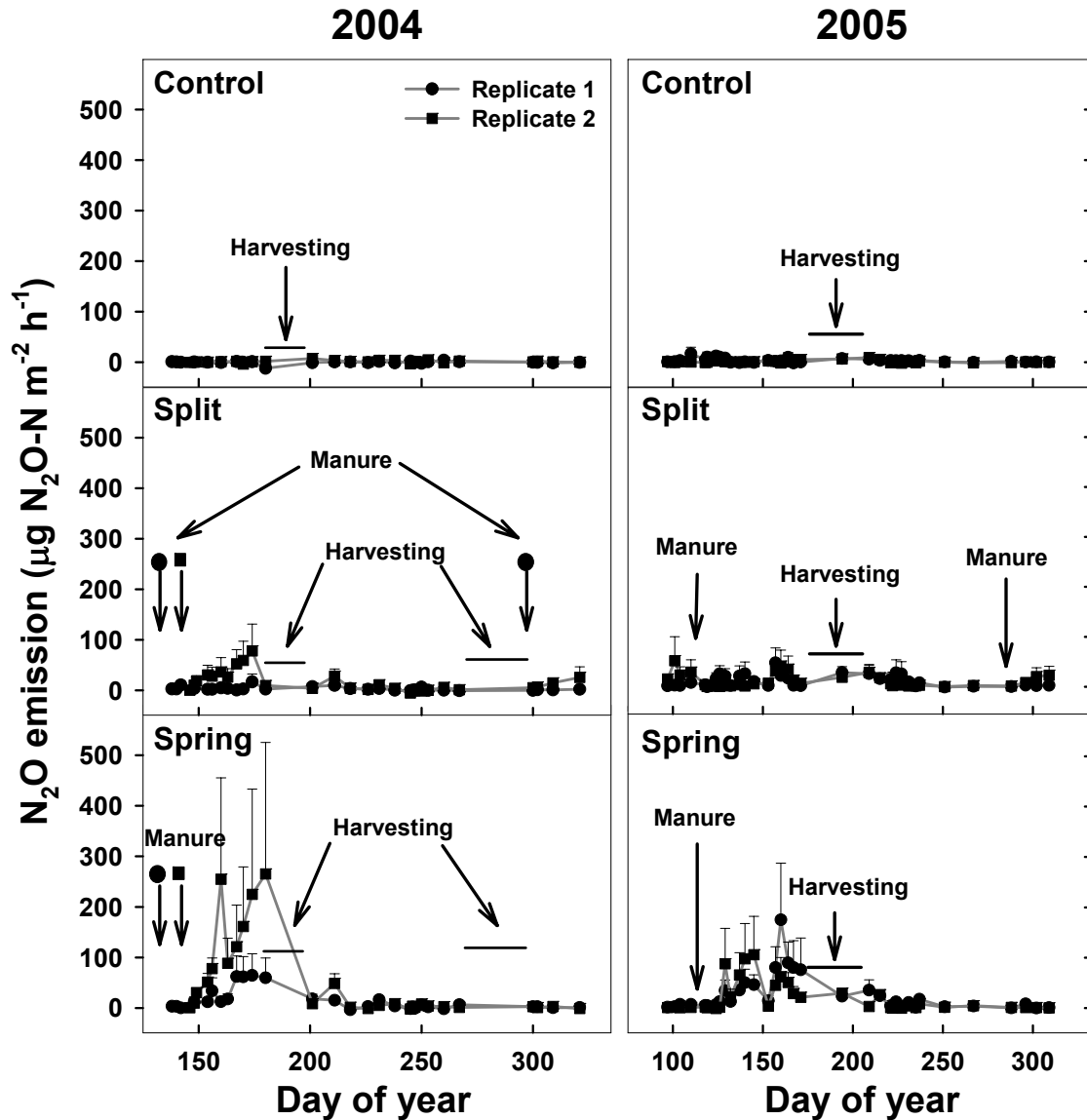


Figure 2.4. Soil nitrous oxide emission from the plot experiment during the 2004 and 2005 growing seasons for Control, Split and Spring manure treatments. Values represent the average of 6 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1 and square points represent Replicate 2 results. Events such as manure application and harvesting are indicated. Harvesting refers to the period of time from forage cutting to baling.

emissions of N₂O, while within plots, the driest sample positions would consistently produce higher N₂O emissions. The higher the emissions produced from the plots, the higher the level of variability. For example on June 28, 2004 (DOY 180), the Spring plot in Replicate 2 produced an average of 265 μg N₂O-N m⁻² h⁻¹ with a CV of 240% as almost all these emissions were produced from one chamber of the six. In comparison, this same plot produced an average of 30 μg N₂O-N m⁻² h⁻¹ with a CV of 72% on May 28, 2004 (DOY 149).

2.4.2.1.2. Methane. Generally, manure treated plots produced more CH₄ than untreated plots (Fig. 2.5). In 2004, the Control plots produced very little CH₄ and the maximum measured emissions from any sample position in the manured plots was over 18 times larger than that from any sample position in the Control plots. In 2005, there were very high emissions of methane in all treatments of the first replicate and consequently, the maximum measured emissions in the manured plots was only 1.9 times greater than that of the Control. In both years, the soil CH₄ emissions that did occur were highly variable both between replicates and within plots. Methane emissions were found only in the wetter replicates, and only the wettest sample positions emitted CH₄. Sample locations in drier areas were found to be net consumers of CH₄, regardless of manure treatment. As with N₂O, there was high variability within plots when there were high CH₄ emissions. For example, on June 4, 2004 (DOY 156), the Spring plot in Replicate 1 produced an average of 233 μg CH₄-C m⁻² h⁻¹ with a CV of 141%.

The temporal pattern of CH₄ emissions from plots behaved differently for the study years (Fig. 2.5). In 2004, CH₄ emissions were produced on some of the plots immediately after spring manure application. This was very different from the case in

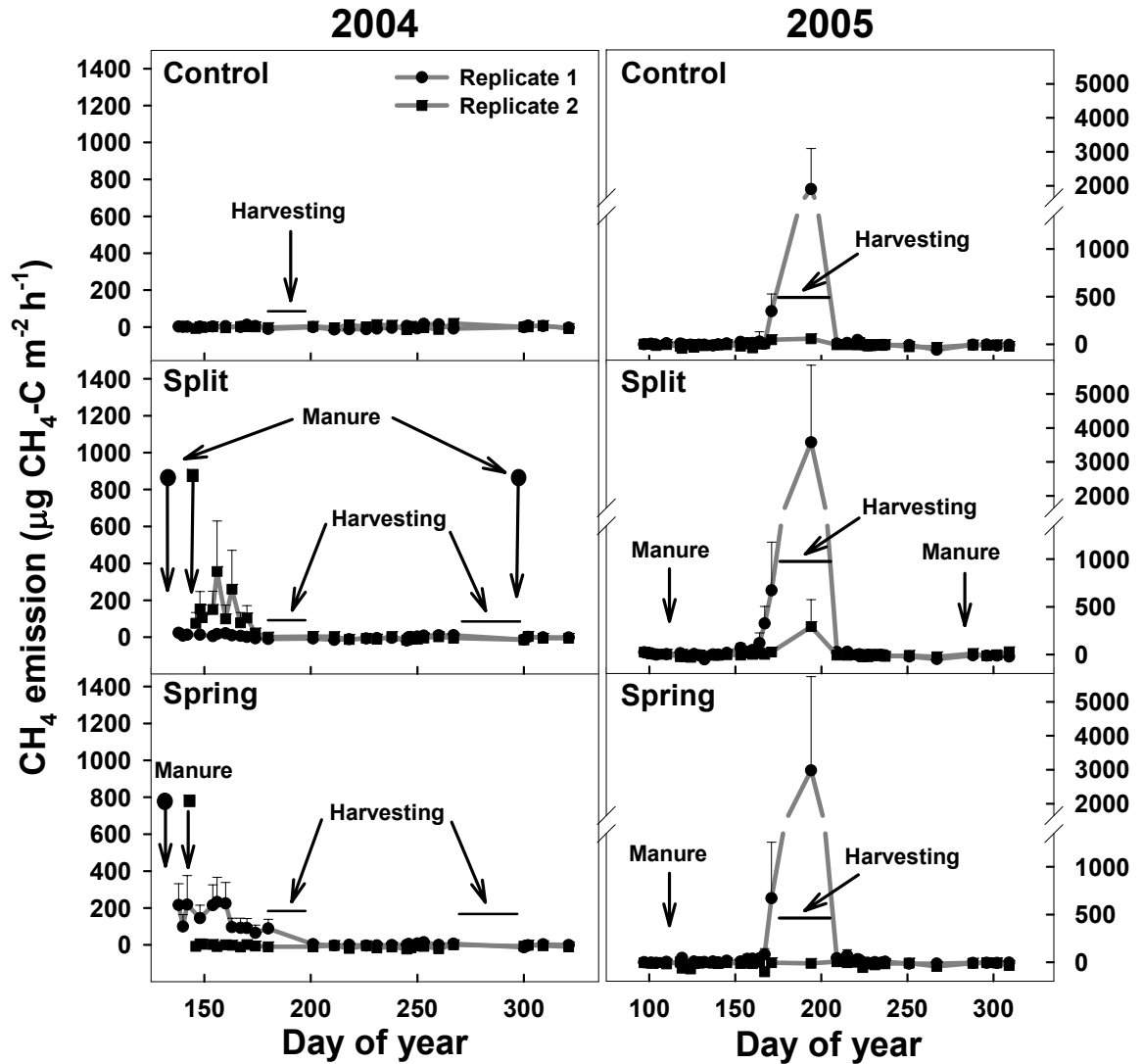


Figure 2.5. Soil methane emission from the plot experiment during the 2004 and 2005 growing seasons for Control, Split and Spring manure treatments. Values represent the average of 6 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1 and square points represent Replicate 2 results. Events such as manure application and harvesting are indicated. Harvesting refers to the period of time from forage cutting to baling. Note differences in scale for 2004 and 2005.

2005, when there was very little CH₄ emission after spring manure application, but a very large CH₄ emission from all treatments (including the Control) in Replicate 1 during the summer. In the two years of the study, there was little CH₄ emission from the plots after the fall manure application to the Split treatments.

2.4.2.1.3. Respired Carbon Dioxide. Similarly to N₂O and CH₄ emissions, soil CO₂ emissions were higher from the manure treated plots than from the control plots (Fig. 2.6). However, the variability of the CO₂ emissions within plots was less than that of N₂O and CH₄ emissions. The variability of CO₂ emissions between replicates was also much less compared to the N₂O and CH₄ emissions. Low variability in CO₂ emissions compared to N₂O and CH₄ emissions was expected, as all sample positions produced CO₂ emissions where as only certain sample positions produced N₂O and CH₄ emissions.

There were some differences in CO₂ emissions between 2004 and 2005, with higher CO₂ emissions in 2005. In general, CO₂ flux patterns increased into the growing season and decreased into fall and winter. Increases in CO₂ emission were also evident after spring manure application in 2004 and 2005, although there is no apparent increase in CO₂ emission after the fall manure applications to the Split application treatments.

2.4.2.2. Tests for Treatment Effects on Gas Emissions.

2.4.2.2.1. Repeated Measures Test. The repeated measures test indicated treatment differences for N₂O emissions and the CO₂ equivalents of cumulative N₂O and CH₄ emissions, however, there were no significant treatment differences for CH₄ emissions (Fig. 2.7). The strongest differences in N₂O emissions occurred soon after spring manure application, with the largest differences during the third GDD period (corresponds to

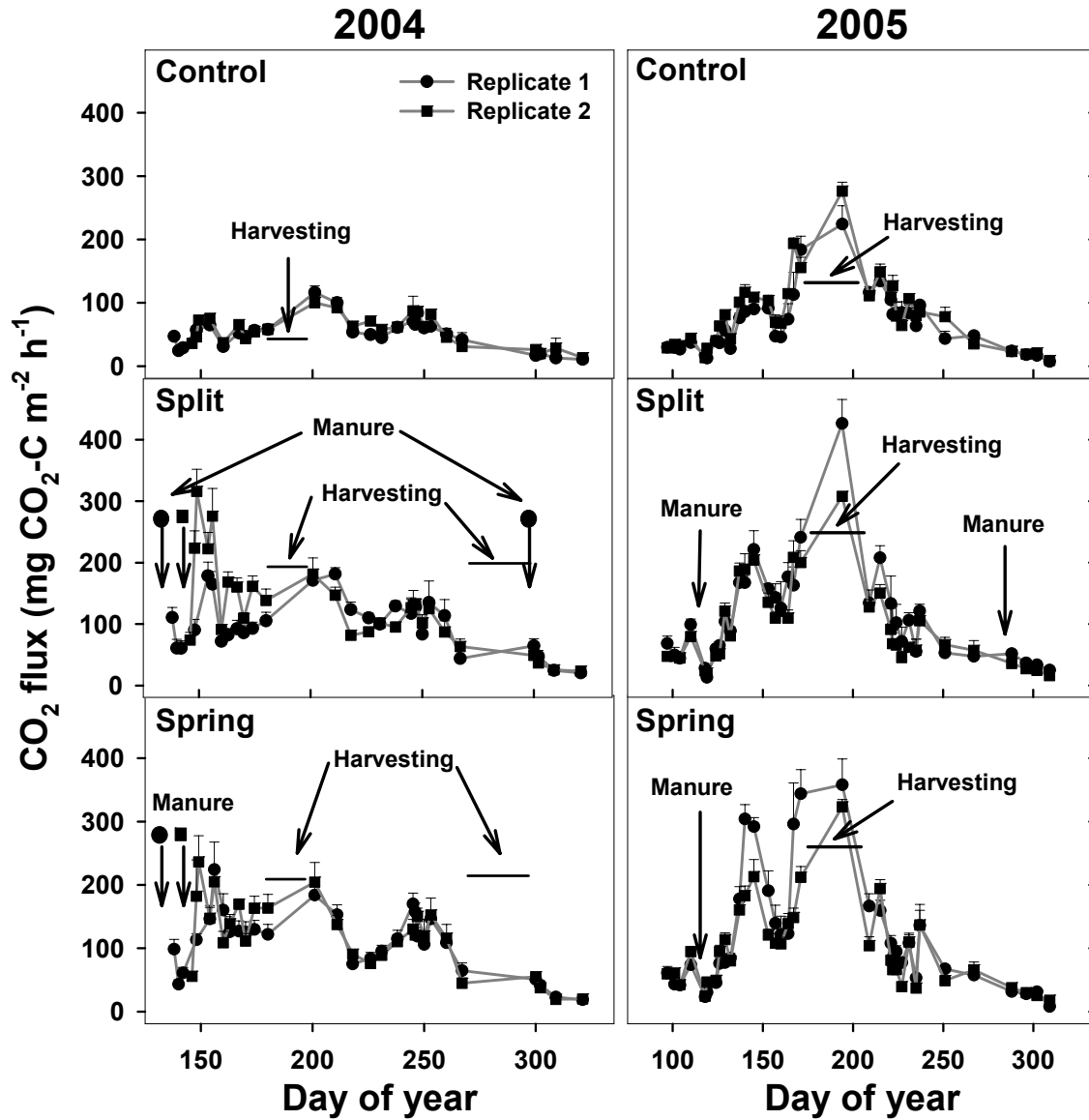


Figure 2.6. Soil respiration (dark chamber CO₂ emission) from the plot experiment during the 2004 and 2005 growing seasons for Control, Split and Spring manure treatments. Values represent the average of 6 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1 and square points represent Replicate 2 results. Events such as manure application and harvesting are indicated. Harvesting refers to the period of time from forage cutting to baling.

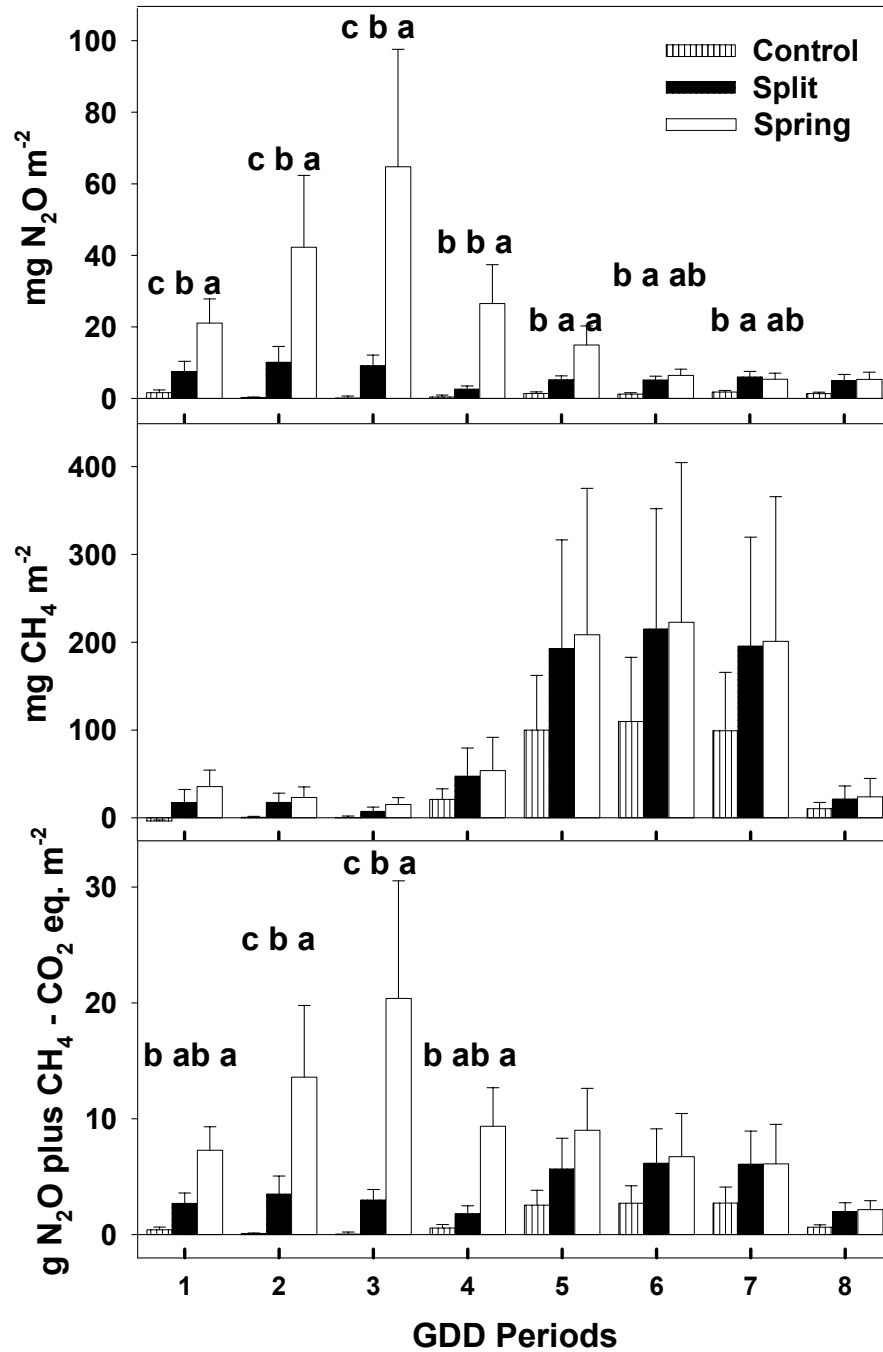


Figure 2.7. Cumulative N₂O, CH₄ and N₂O plus CH₄ equivalent CO₂ emissions for 8 Growing Degree Day (GDD) periods in 2004 and 2005. Mean values followed by different letters in the same period are significantly different at P≤0.05 (Tukey's test) (based on log-transformed data). Values are the means of 24 sample positions, over two replicates and two years. See text for details of growing degree days.

between June 21 (DOY 173) and July 4 (DOY 186) in 2004, and between June 2 (DOY 153) and June 13 (DOY 164) in 2005. The first seven GDD periods show at least one of the manure treatments had significantly higher N₂O emissions than the Control treatment. Of particular interest is that during the first four GDD, the Spring treatment had significantly higher N₂O emissions than the Split treatment. Although there were no significant treatment differences for CH₄ emissions, the two manure treated plots had consistently higher emissions than the Control treatment. No analysis can be made for effects of fall manure application, as the GDD periods did not encompass emissions this late in the season.

When N₂O and CH₄ emissions were combined as CO₂ equivalents, there were less GDD periods with significant treatment differences than when comparing N₂O emissions alone. This is likely due to an increase in variability of the data compared to the N₂O data, due to the high variability of the CH₄ emissions. For combined CO₂ equivalents, the first four GDD periods showed at least one of the manure treatments having significantly higher emissions than the Control treatment. The first and fourth GDD periods showed increased emissions between the Spring and Control treatments, while the second and third GDD periods showed increasing emissions from the Control, Split and Spring treatments. From this analysis, it appears that N₂O had a greater influence on the combined CO₂ equivalents than CH₄.

2.4.2.2.2. Cumulative Gas Emissions. The tests of significance for cumulative greenhouse gas emissions of the two replicates and years reflected the results of the repeated measures test (Table 2.4). Over all replicates and study years, both manure treatments produced significantly ($P \leq 0.01$) higher cumulative N₂O emissions than the

Table 2.4. Cumulative greenhouse gas emissions from treatments and replicates for 2004 and 2005. Mean values for treatments across Replicates and Years followed by the same letter are not significantly different (based on log transformed data) (Fisher's Protected LSD test, $P \leq 0.10$). Values for each replicate (Rep 1 and Rep 2) represent the mean of 6 sample positions. Values for average and standard deviation represent means of 12 sample positions, 6 sample positions for each replicate over the two years of the study.

N₂O						
	2004		2005		Mean*	SEM
	Rep 1	Rep 2	Rep 1	Rep 2		
	mg N₂O-N m⁻²				P\leq0.10	
Control	-3.97	7.59	13.24	9.88	6.69 c**	1.70
Split	12.87	55.78	50.44	51.31	42.60 b	7.94
Spring	62.19	196.04	123.08	98.06	119.84 a	40.77

CH₄						
	2004		2005		Mean*	SEM
	Rep 1	Rep 2	Rep 1	Rep 2		
	mg CH₄-C m⁻²					
Control	-9.9	9.0	955.3	-5.3	237.3	165.8
Split	-9.1	97.8	1867.0	123.9	519.9	334.8
Spring	169.3	-31.0	1609.8	-81.7	416.6	377.5

CO₂ equivalents (combined N₂O and CH₄ emissions)						
	2004		2005		Mean*	SEM
	Rep 1	Rep 2	Rep 1	Rep 2		
	g CO₂ m⁻²				P\leq0.01	
Control	-2.03	3.34	33.24	4.67	9.80 b***	4.74
Split	6.13	30.72	76.94	28.46	35.56 a	11.09
Spring	35.49	94.82	105.10	45.47	70.22 a	21.39

* Statistical analyses performed on entire data set (both replicates and years) using log-transformed data.

** Differences between Control and both manure treatments significant at $P \leq 0.01$, difference between Full and Split manure treatments significant at $P \leq 0.10$.

*** Differences between Control and both manure treatments significant at $P \leq 0.01$.

Control. The Spring treatment produced higher cumulative N₂O emissions than the Split treatment with a significance value of $P \leq 0.10$. As for CH₄ emissions, there were no significant treatment differences, and variability was very high between the replicates and years. However, higher cumulative CH₄ emissions did tend to occur in the manure treated plots. Some plots were shown to be net consumers of CH₄, such as the Spring plot of Replicate 2.

When N₂O and CH₄ emissions were combined as CO₂ equivalents, N₂O was the more important of the two GHG's. Lower N₂O emissions resulted in lower levels of CO₂ equivalents produced in the Spring treatment in 2005 compared to 2004. Over all replicates and study years, there were significantly ($P \leq 0.01$) higher emissions of CO₂ equivalents in the manure treated plots compared to the control.

2.4.2.3. Rainfall, Air Temperature, Soil Temperature and Soil Moisture. The two study years were both much wetter than normal, although the distribution of rainfall was quite different for the two years. In 2004, there were many cool, cloudy, rainy days, while 2005 was warm with several large rainstorms (Fig. 2.8). In 2004, the research site received 521 mm of precipitation between May 16 (DOY 137) and November 4 (DOY 309), compared to normal precipitation of 356 mm (Steinbach, Manitoba weather station data). In 2005, the site received 578 mm of precipitation between April 14 (DOY 104) and November 1 (DOY 305), with normal of 401 mm. Conditions were very wet during the spring of 2004, and the soil was saturated in places when manure was applied. These wet conditions persisted into June. It was also wet during the fall manure application in 2004, though temperatures were cooler than in the spring. The weather in 2004 was cooler than 2005 with average temperatures (June to August) of 15.6 and 18.6,

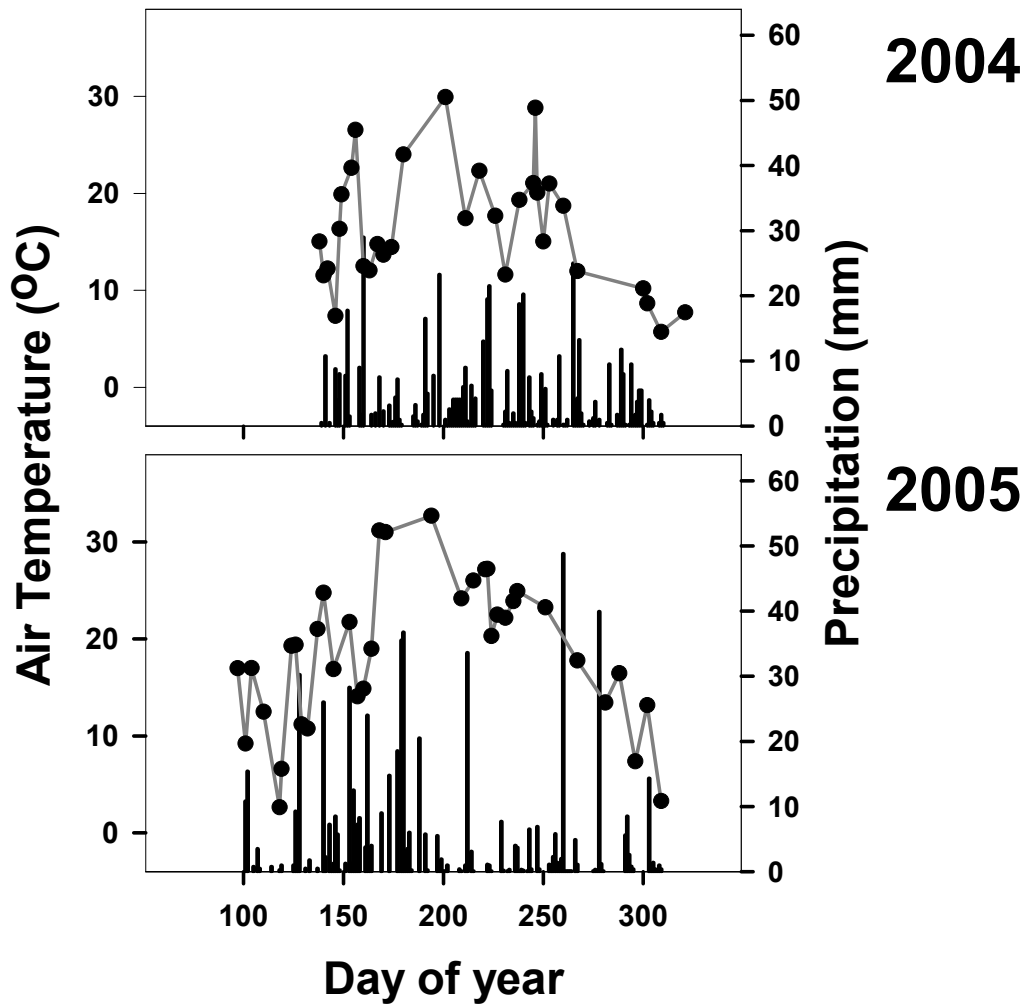


Figure 2.8 Air temperature at time of gas sampling of all plots and also daily rainfall for the study periods in 2004 and 2005.

respectively. In 2005, conditions were very wet, although rainfall came in much larger events with hot, dry periods in between. The spring of 2005 was dry (only 28 mm of precipitation between April 7 (DOY 97) and May 5 (DOY 125) allowing for application of manure on April 26, 2005 (DOY 116), which was two to four weeks earlier than in 2004. In comparison to 2004, the emissions of N₂O were delayed after spring manure application in 2005, coinciding with cool and dry conditions. In addition, there was very little CH₄ emission after manure application during the spring of 2005, but the very high methane emissions during the summer corresponded to a period of time that was very wet and hot. Conditions were dry for fall manure application in 2005. Soil temperatures at 2.5 cm depth for the 2004 and 2005 study periods followed the same patterns as air temperature (Fig. 2.9).

The abnormally high precipitation in both study years created wet soil conditions for long periods. Water table elevation shown in Figure 2.10 is for a location immediately to the northeast of the study site. This location was typical of a low lying area at the site, where the water table would come to the surface during wet periods. Generally, most of the N₂O emissions were produced at the drier sample positions in the plots. The highest emissions of N₂O that occurred during this study were in the Spring treatment of replicate 2, the driest plot of all (Fig. 2.11). Saturated areas of the plots would produce CH₄ emissions. In most plots, soil moisture would generally increase from the alleyway separating Replicate 1 from 2 towards the outside edge of the site (either western or eastern edge of the site). An exception was the Control Replicate 2 plot, which did not seem to have a clear moisture gradient from one edge of the plot to the next.

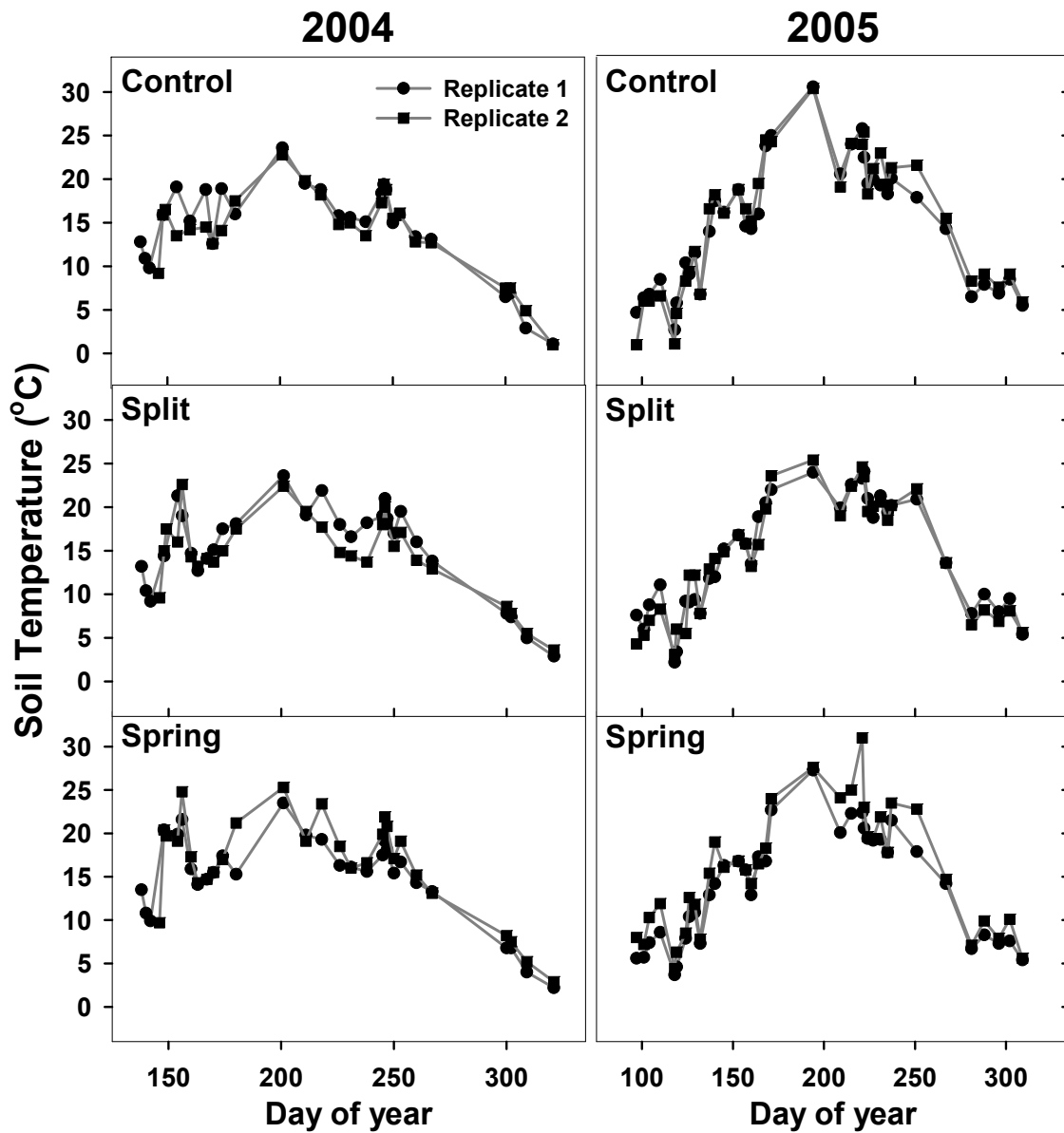


Figure 2.9 Soil temperatures at 2.5 cm at time of gas sampling in 2004 and 2005 for the Control, Split and Spring manure treatments. Values represent the average of 6 sample positions.

Note: Standard error of the mean not shown as variance was low.

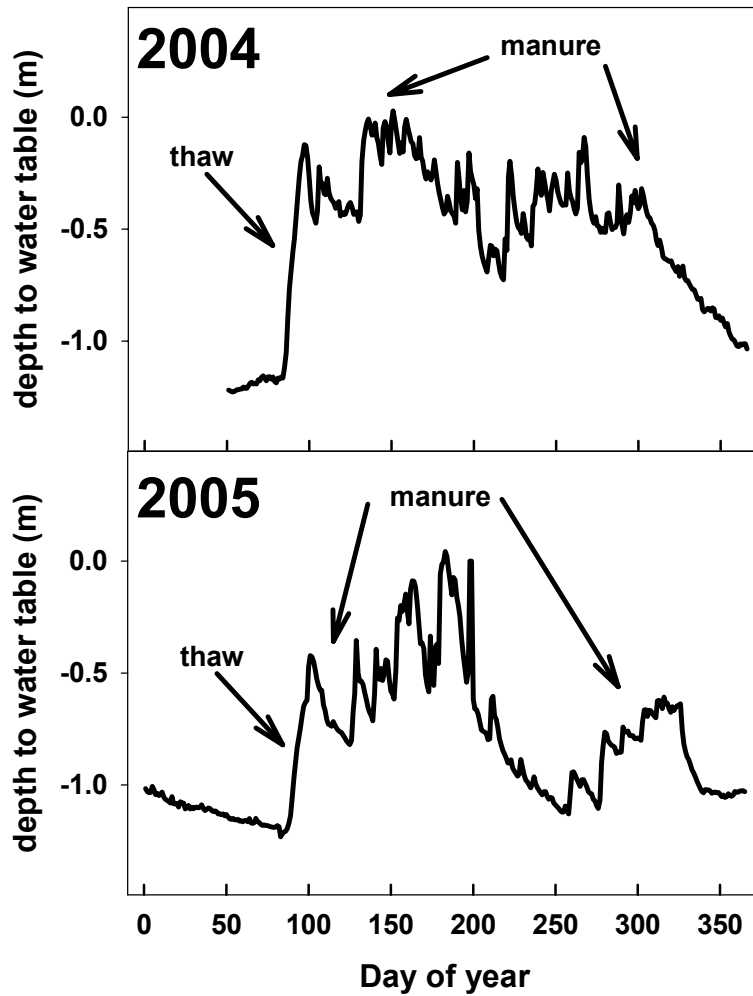


Figure 2.10. Depth to water table at the La Broquerie Pasture Project Research site (Data courtesy of Dr. Graham Phipps, Manitoba Water Stewardship).

Note: Transducer located at NE edge of site began recording data on February 20, 2004.

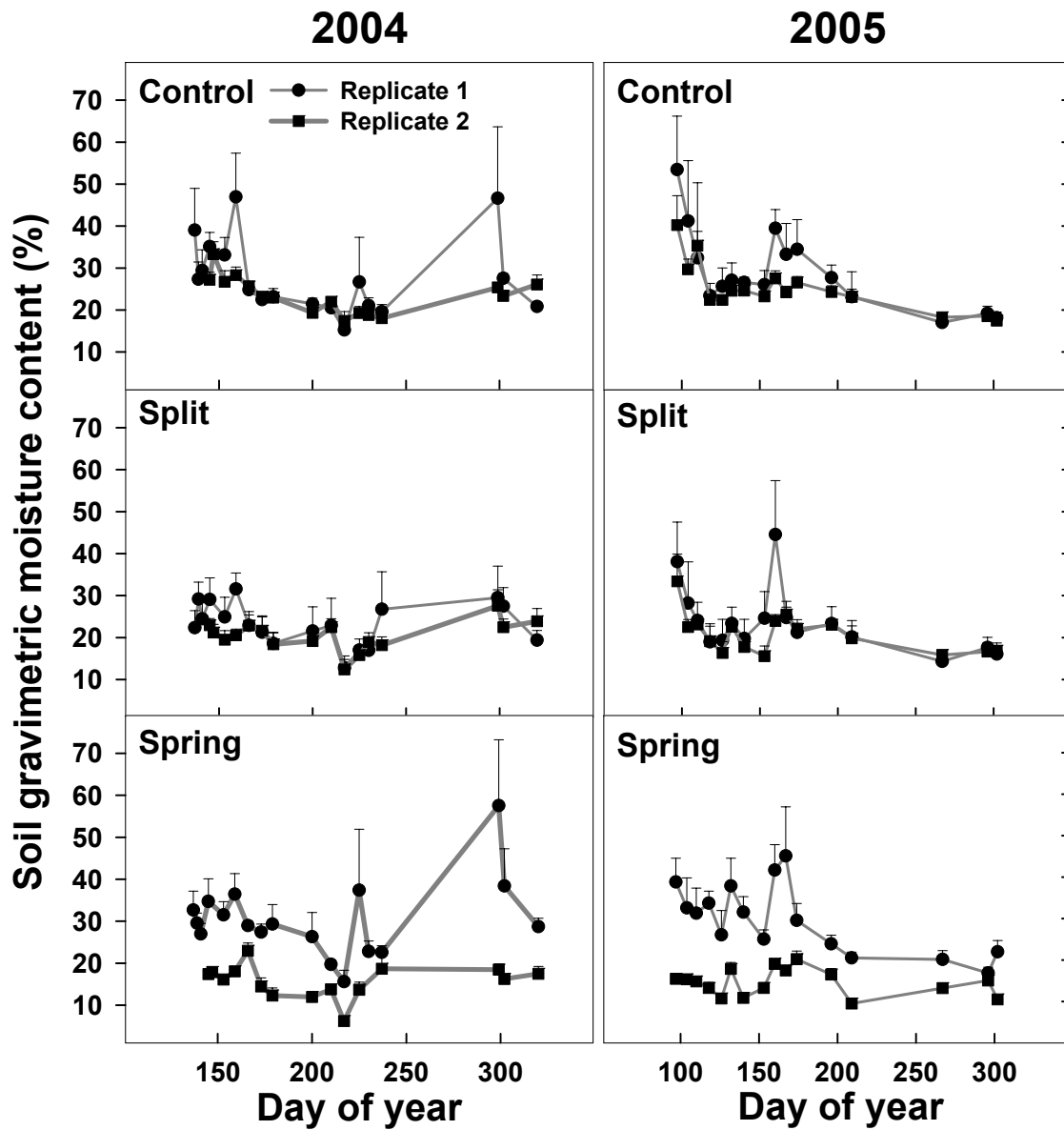


Figure 2.11. Soil gravimetric moisture content from the plot experiment during the 2004 and 2005 growing seasons for the Control, Split and Spring manure treatments. Values represent average of 6 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1 and square points represent Replicate 2.

2.4.2.4. Soil Nutrients – Ammonium and Nitrate. Generally, soil NH_4^+ levels were highest following manure application and then dropped to Control treatment levels within 5 weeks (Fig. 2.12). The variability of soil NH_4^+ levels between replicates for the spring of 2004 can be explained by the fact that we had two manure application dates that spring. Manure was applied earlier to Replicate 1, and there was heavy precipitation (May 11 snowfall and rainfall) after the application which may have caused some loss of nutrients before soil samples could be collected. It was observed that soil NH_4^+ concentrations varied within plots with higher concentrations in drier areas compared to wetter areas (data not shown). There was also a tendency for higher soil NH_4^+ in manured treatments of 2004 than 2005; this may be the result of a higher rate of available N applied to the site (Table 2.1) during the spring of 2004 compared to 2005. There was no evident increase in soil NH_4^+ concentration after both fall manure applications to the Split plots compared to the Control or Spring treatments.

Similar to NH_4^+ concentrations, soil nitrate (NO_3^-) levels were generally higher in the manure treated plots than in the Control plots (Fig 2.13). The mean concentration of NO_3^- in the Control plots was never higher than $4.0 \text{ mg NO}_3^- \text{ kg}^{-1}$ dry soil. Fall manure application to the Split treatment did not result in an appreciable increase in soil NO_3^- . Generally, soil nitrate concentrations increased as soil NH_4^+ concentrations decreased in the manure treated plots. This is an indication that nitrification is consuming soil NH_4^+ and producing NO_3^- . Also, the spatial variability of NO_3^- concentrations was seemingly impacted by the soil moisture gradients present in most plots, with higher concentrations of NO_3^- found in drier areas of the plots (data not shown). For example, on June 9, 2005 (DOY 160), there was a 5 times difference in soil NO_3^- concentration between two (dry

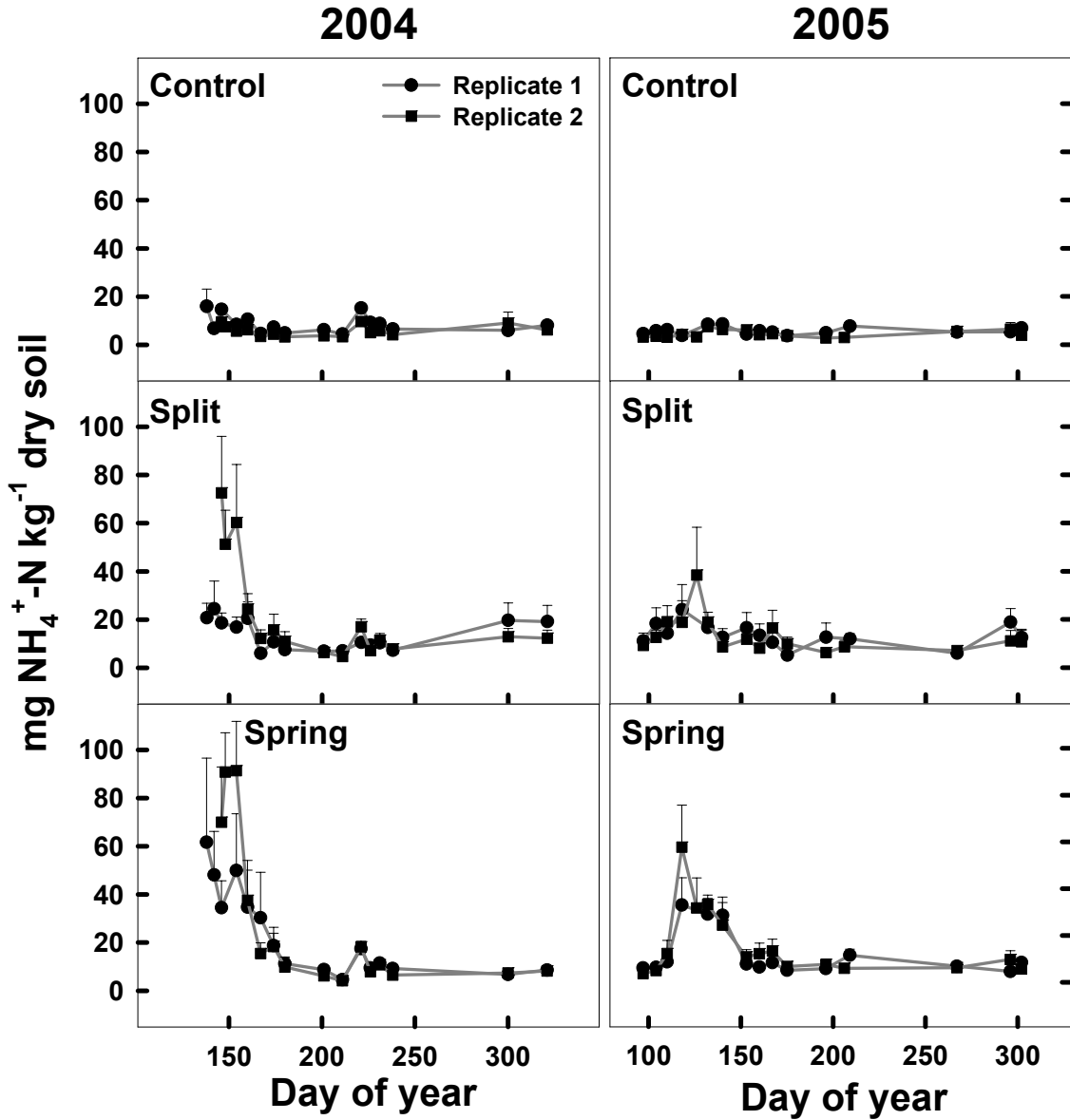


Figure 2.12. Soil ammonium concentration from the plot experiment during the 2004 and 2005 growing seasons for the Control, Split and Spring manure treatments. Values represent average of 6 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1 and square points represent Replicate 2.

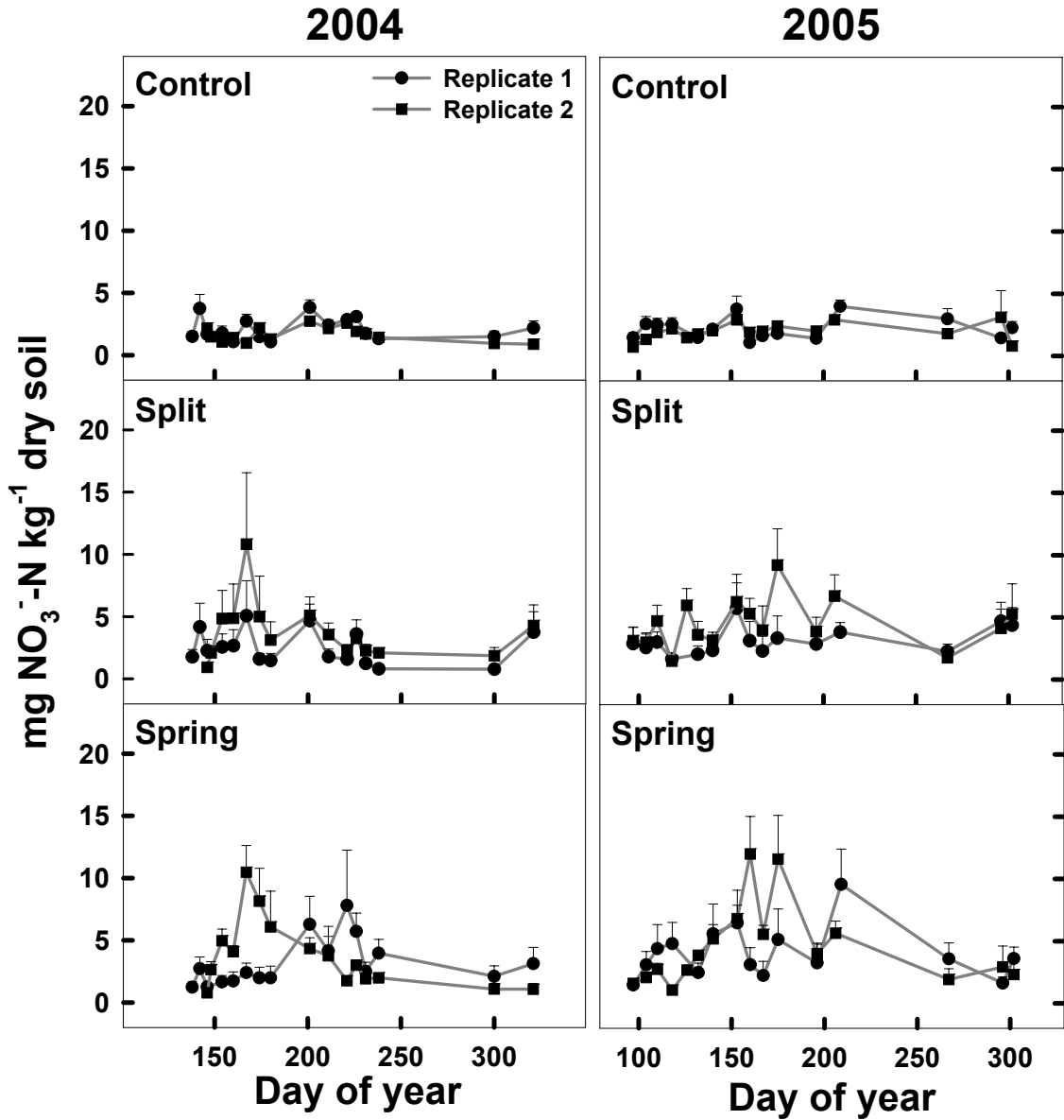


Figure 2.13. Soil nitrate concentration from the plot experiment during the 2004 and 2005 growing seasons for Control, Split and Spring manure treatments. Values represent average of 6 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1 and square points represent Replicate 2.

and wet) sample positions on the Spring plot in Replicate 2.

2.4.2.5. Relation of Measured Parameters to Gas Emissions.

2.4.2.5.1. Correlation and Scatterplots. Most of the Spearman correlations between variables were not very strong, with many correlations having low goodness-of-fit (r -value) (Table 2.5). Generally $P \leq 0.0001$ indicate strong correlations using Spearman rank analysis. Nitrous oxide emissions were most strongly correlated to NO_3^- and slightly less so to CO_2 emissions. Methane emissions were most strongly correlated to soil moisture and were negatively correlated to soil NO_3^- .

For this analysis, the very high CH_4 emission data from the summer of 2005 (July 13, DOY 194) was removed in order to observe the relationships in the rest of the data. This decision was made because it was evident that these large CH_4 emissions did not relate to normal conditions driving methanogenesis. Nitrous oxide emissions mostly occurred when the soil had a gravimetric moisture content between 9 and 34% (Fig. 2.14a). Soil NO_3^- concentrations were low when gravimetric moisture content was above 40%, and was highest between 10 and 20% (Fig 2.14b). Nitrous oxide emissions were produced at very low NO_3^- concentrations, indicating that nitrification may have produced some of the N_2O emissions. Methane emissions occurred when soil gravimetric moisture content was above about 20% (Fig. 2.14d) and appeared to be negligible above 5 mg NO_3^- -N kg^{-1} dry soil (Fig. 2.14e). Methane was also controlled by soil temperature, with emissions occurring when soil temperature at 2.5cm depth was above 8°C (Fig. 2.14f). Higher N_2O emissions were associated with increasing soil NO_3^- concentration (Figs. 2.4 and 2.13), however, there were many times when soil NO_3^- was high and there were negligible N_2O emissions (not shown).

Table 2.5. Spearman Correlation Coefficients and probability level for greenhouse gas data collected after the application of manure in the spring (both replicates and both years). Correlation coefficients with $P \leq 0.0001$ are shown in bold.

	N_2O	CH_4	CO_2	NH_4^+	NO_3^-	gravimetric moisture content	soil temperature
N_2O	1.00000						
CH_4	-0.03553 0.2246	1.00000					
CO_2	0.38782 ≤ 0.0001	0.06824 0.0196	1.00000				
NH_4^+	0.14316 ≤ 0.0001	-0.0071 0.8084	0.06859 0.0190	1.00000			
NO_3^-	0.41213 ≤ 0.0001	-0.23461 ≤ 0.0001	0.22230 ≤ 0.0001	0.33776 ≤ 0.0001	1.00000		
gravimetric moisture content	-0.17107 ≤ 0.0001	0.42442 ≤ 0.0001	-0.06618 0.0236	-0.13246 ≤ 0.0001	-0.33040 ≤ 0.0001	1.00000	
soil temperature	0.17016 ≤ 0.0001	0.09438 0.0012	0.70316 ≤ 0.0001	-0.09928 0.0007	0.16709 ≤ 0.0001	-0.15084 ≤ 0.0001	1.00000

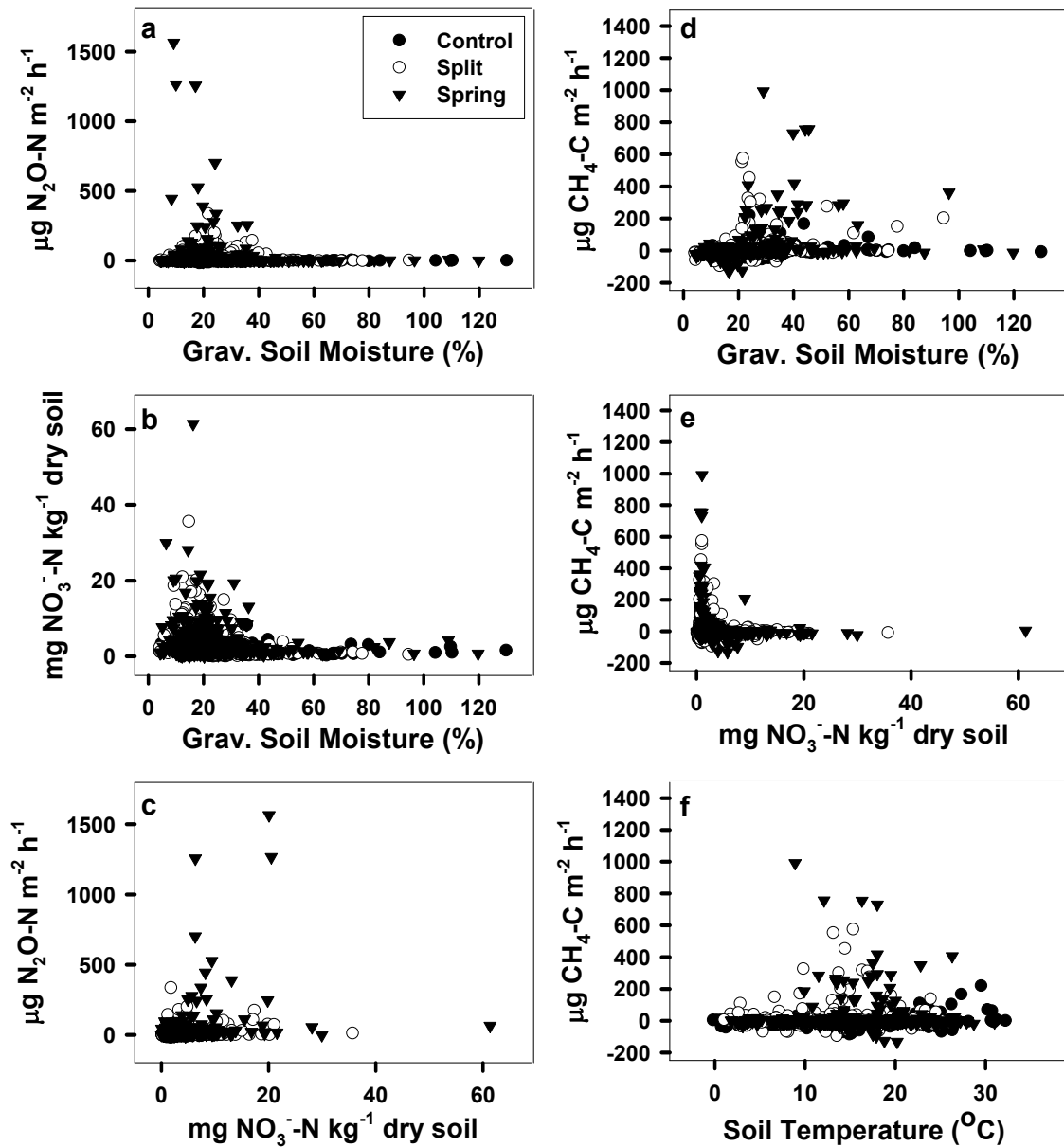


Figure 2.14. Select scatterplots of soil parameters and N_2O and CH_4 emissions. Values are for individual sample positions and sample dates for both study years of 2004 and 2005. Values in 2005 having very high CH_4 emissions are not shown. (a) Gravimetric moisture content vs. N_2O emission, (b) Gravimetric moisture content vs. soil NO_3^- , (c) Soil NO_3^- vs. N_2O emission (d) Gravimetric moisture content vs. CH_4 emission, (e) Soil NO_3^- vs. CH_4 emission, (f) Soil temperature vs. CH_4 emission.

2.4.3. Transect Study

2.4.3.1. Weather Conditions. During the gas sampling period for this experiment (June 2 to June 13, 2005) there was a total of 86.75 mm, with major rainfalls on June 3 (28.25 mm), June 5 (12.5 mm), June 7 (7.25 mm), June 8 (8.25 mm) and June 12 (24 mm). Although the study site was relatively dry at the start of this experiment, the rain produced much wetter soil conditions for the June 6 and June 13 gas sampling dates than for June 2. The water table rose to the soil surface in places, submerging some of the static-vented chambers at the western end of the transects and making soil sampling difficult. Air temperatures at the time of sampling were 21.4, 15.0 and 18.6°C on June 2, 6 and 13, respectively.

2.4.3.2. Gas Emission.

2.4.3.2.1. Nitrous Oxide. On June 2, there was very little N₂O emissions from both the Control and Spring treatment transects (Fig. 2.15). On June 6, after the heavy rainfalls of June 3 and 5, there were large N₂O emissions from the drier areas of the Spring treatment transect. Even larger N₂O emissions occurred on June 13 after more rain had fallen and the temperature had risen. Smaller N₂O emissions also occurred from some of the sample positions in the drier areas of the Control transect on June 6 and June 13. Very little N₂O emissions were measured in the wetter areas of both transects on the three sampling days.

2.4.3.2.2. Methane, Soil Moisture, Soil Nutrients and Biomass. Methane was emitted from the transects on all three sampling dates (Fig. 2.15). However, CH₄ emissions become progressively larger towards the third sampling date, with more CH₄ emissions from the Spring transect than the Control transect. There was also a large CH₄ emission

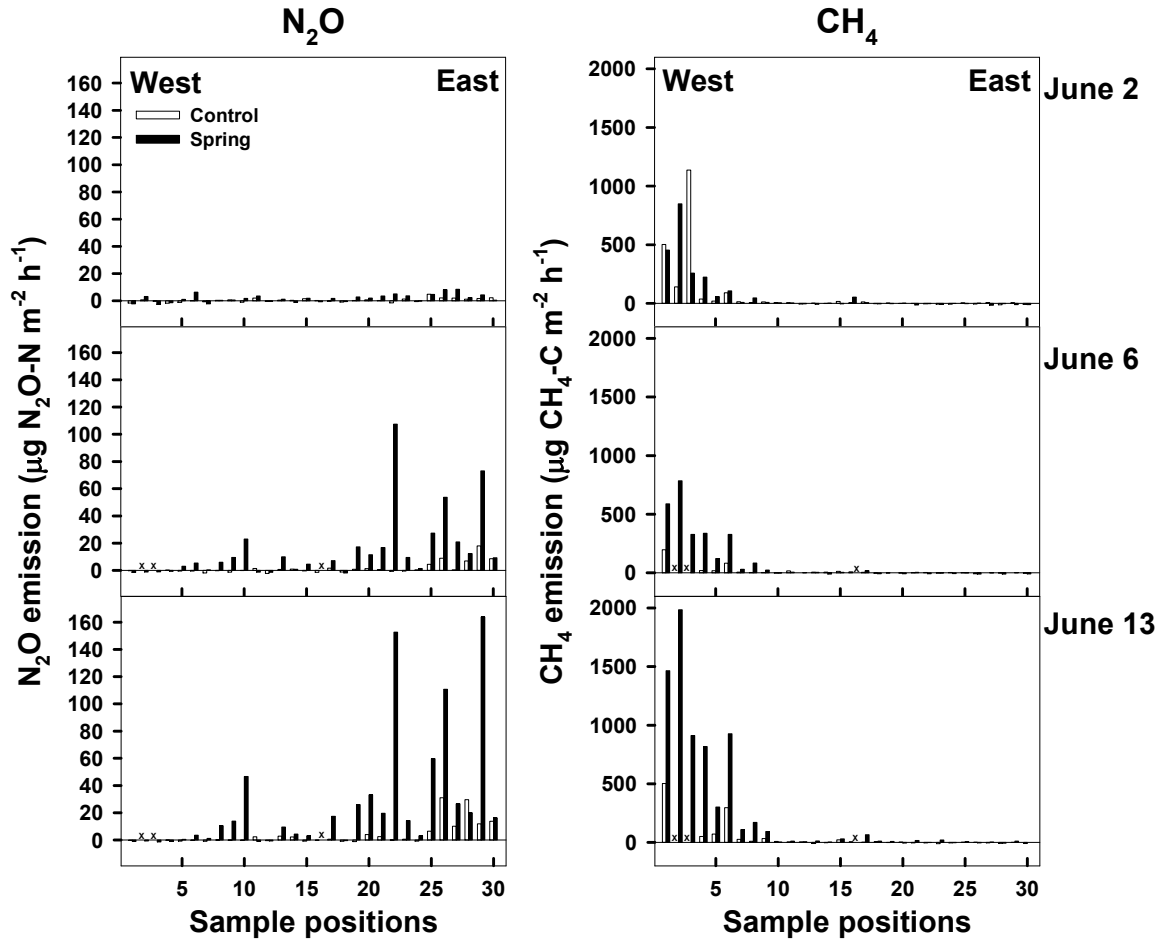


Figure 2.15. Soil N_2O and CH_4 emission for the transect experiment for June 2, 6 and 13, 2005 sample dates. Control transect is represented by clear bars, Spring transect is represented by black bars. Sample positions are arranged from East to West. Missing data for three sample positions due to submerged gas collars are indicated by an 'x'.

from one of the control sample positions on June 2, however this chamber was submerged in surface water on the following sampling days and was not sampled. As with N_2O , a distinct pattern in the distribution of CH_4 emissions across the transects was evident. However, this pattern was much more distinct, with CH_4 only being produced in the wettest areas of the transects. At other sample locations, CH_4 emissions were actually slightly negative.

There are two apparent trends for moisture content of the soils collected along the transects (Fig. 2.16). The first is that on both dates when soil samples were collected, the soils became progressively wetter from the eastern to the western reaches of the transects. The second is that the soil was wetter across the transects on June 13 compared to June 2.

Generally, both NH_4^+ and NO_3^- levels were higher in the Spring treatment transect than in the Control transect (Fig 2.16). There was a little more NH_4^+ in the soil at the driest areas of the transect, and there was less NH_4^+ in the soil on June 13 than on June 2. There was a more obvious localization of higher soil NO_3^- concentrations at the drier reaches of the transects, especially in the Spring treatment transect. This localization of soil NO_3^- corresponds to the area where N_2O emissions were highest. The concentration of NO_3^- in the soil decreased from June 2 to June 13, especially in the drier areas of the Spring treatment transect.

On June 24, 2005, below- and above-ground plant biomass samples were taken next to the gas collection positions along the transects (Fig. 2.17). Samples were lost during handling for positions 11 and 14 from the Control transect and position 24 from the Spring treatment transect. Generally, both the below- and above-ground biomass from the Spring treatment transect were higher than that from the Control transect. Root

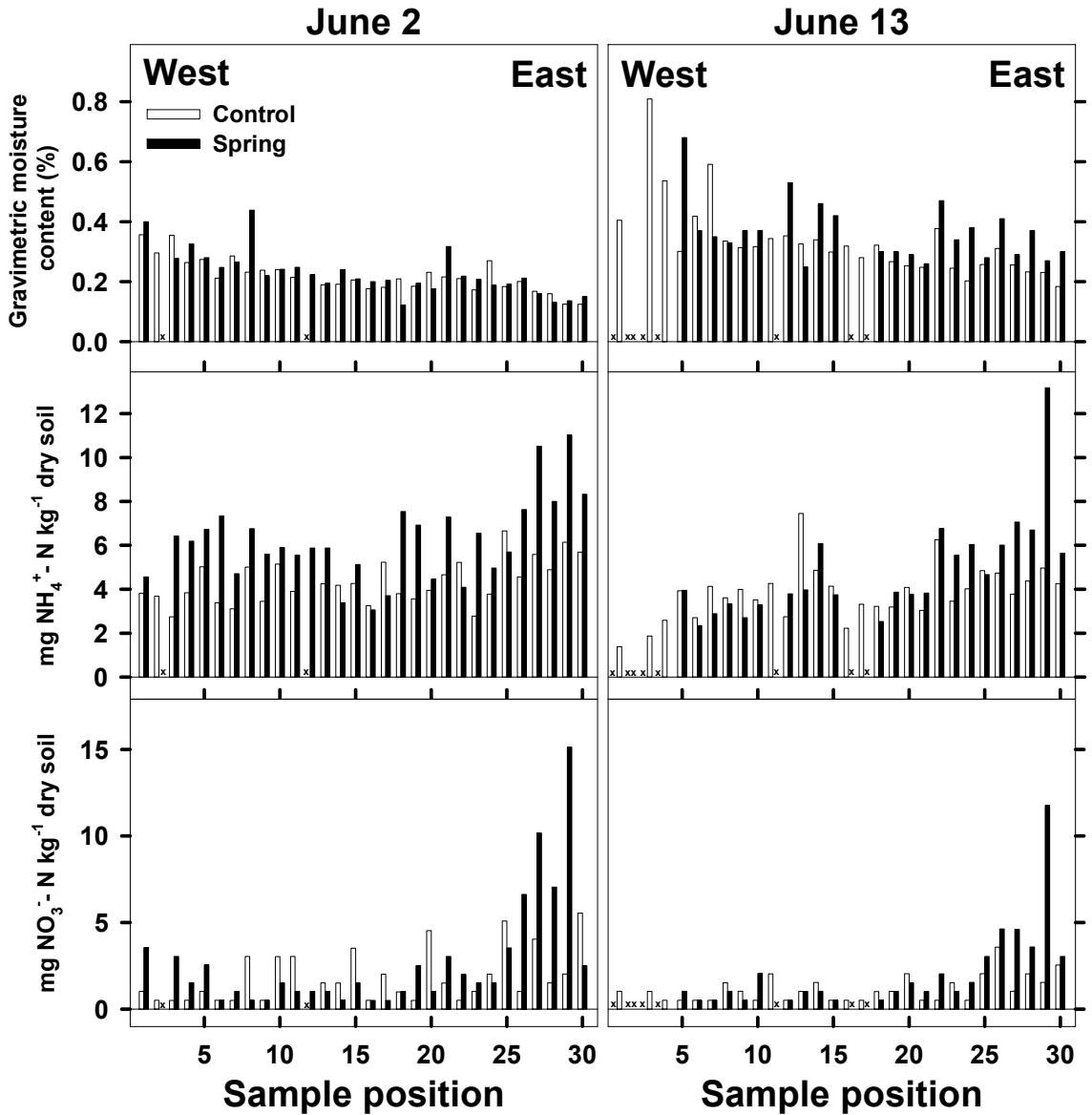


Figure 2.16. Gravimetric moisture content, soil ammonium and soil nitrate concentrations for the transect experiment from June 2nd and 13th, 2005 sample dates. Control transect is represented by clear bars and Spring treatment transect is represented by black bars. Sample positions are arranged from the East to West and were spaced 9 m apart. Missing data due to saturated soil conditions are represented by an 'x'.

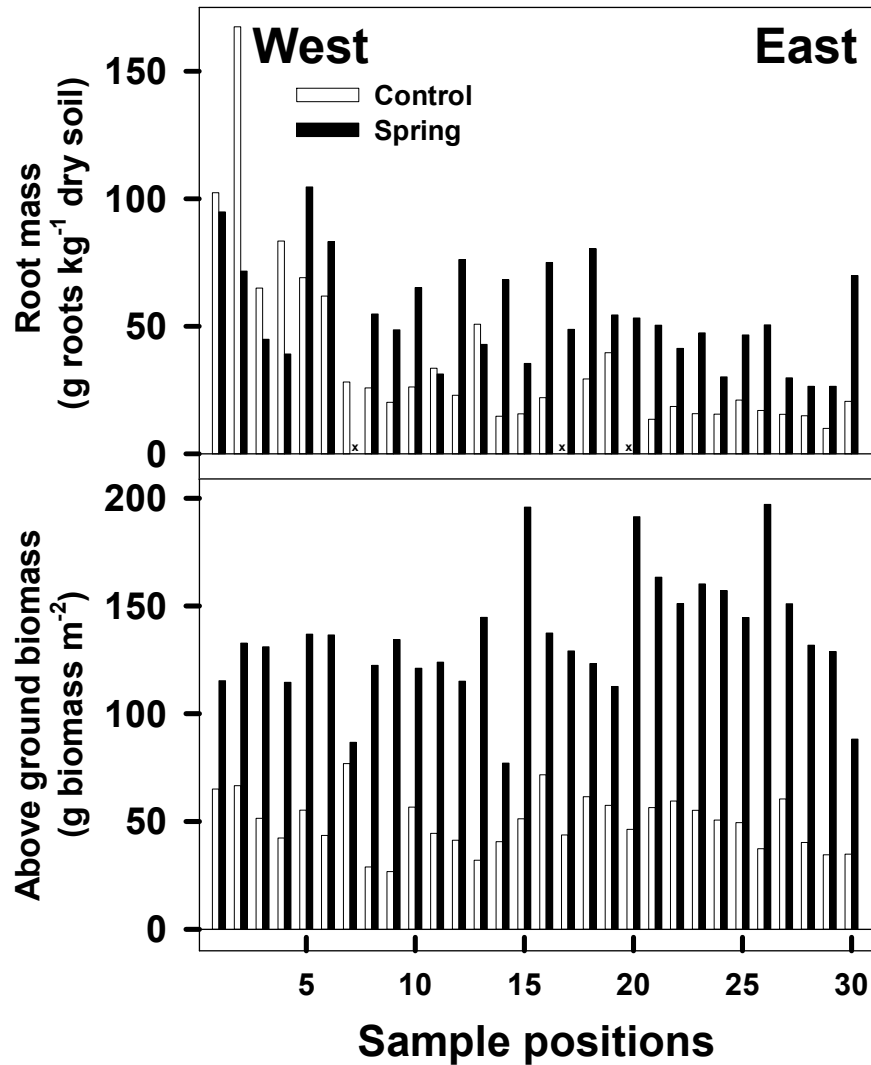


Figure 2.17. Root mass (from 5 cm deep soil cores) and above ground biomass collected on June 24, 2005 from the transect experiment. Control transect is represented by clear bars and Spring treatment transect is represented by black bars. Missing root mass data are indicated by an 'x'.

biomass appeared to be higher in the Control transect than in the Spring treatment transect at the far western edge of the plots. Roots below 5 cm were not collected with the cores thus my determinations of roots do not include potential increase in rooting depth with manure application. The effect of manure treatment on above-ground biomass was apparent across all sample positions along the transects with biomass being at least twice in the Spring than Control treatment. Above-ground biomass was higher in the drier areas of the manure-treated transect.

2.4.3.3. Effect of Soil Moisture on Gas Emissions. Soil moisture content was a factor effecting N₂O and CH₄ emissions over the transects for the two sample dates, June 2 and June 13, 2005, when both gas and soil were analyzed (Figure 2.18*a* and *b*). Two sample positions on the Control treatment transect produced N₂O emission rates that were greater than other sample positions. The emission rates were about 25 µg N m⁻² h⁻¹ for these positions with soil moisture ranging from 0.23 to 0.31 g H₂O g⁻¹ dry soil (Fig. 2.18*a*). In contrast, eight sample positions had N₂O emissions of 25 µg N m⁻² h⁻¹ or greater for the Spring treatment transect with moisture ranging from 0.25 to 0.45 g H₂O g⁻¹ dry soil (Fig. 2.18*b*). These larger incidents of N₂O emission all occurred on June 13. Moisture content was not the only factor determining N₂O emissions as sample positions with moisture contents within the ranges stated above did not emit appreciable N₂O. Three sample positions in the Control transect produced four events with appreciable CH₄ emission (> 250 µg CH₄-C m⁻² h⁻¹) over the two sampling days compared to other positions (Fig. 2.18*b*). Moisture ranged from 0.35 to 0.42 g H₂O g⁻¹ dry soil for the positions producing CH₄. Six sample positions in the Spring transect produced 10 points with appreciable CH₄ emission over the two sampling days when moisture content was

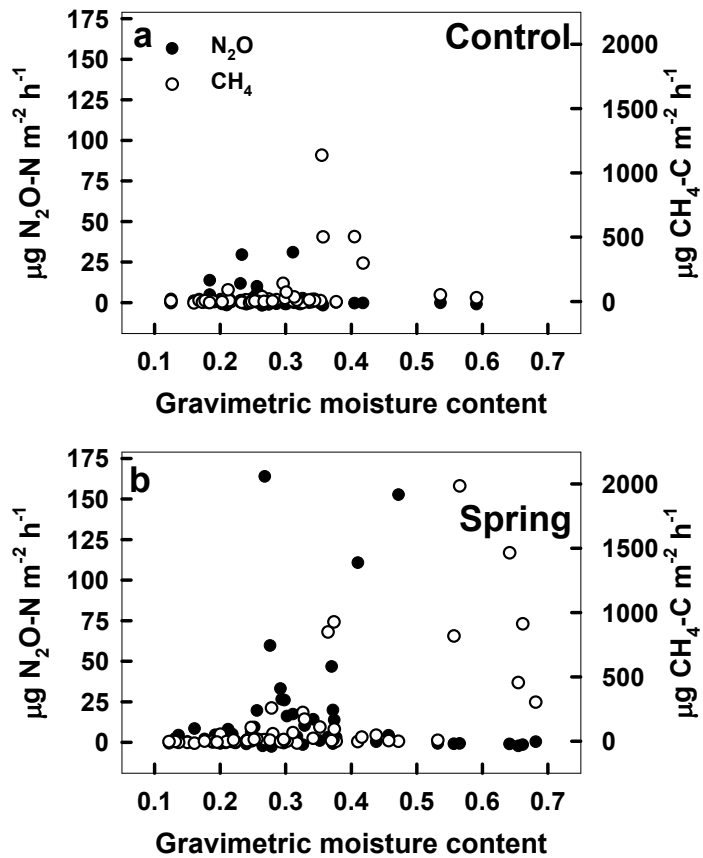


Figure 2.18 Scatterplots of nitrous oxide (N₂O) and methane (CH₄) emissions to gravimetric moisture content for June 2 and 13, 2005 sample dates in the Control and Spring treatment plots of the Transect experiment.

greater than $0.28 \text{ g H}_2\text{O g}^{-1}$ dry soil. This data does not clearly demonstrate that CH_4 emission only occurred on saturated soil, as it was impossible to determine gravimetric moisture content from the saturated areas. This is due to water being pushed out of the soil during sampling, as well as draining from the soil once the soil was pulled from the ground. However, this data does demonstrate that CH_4 was typically emitted in wetter conditions than N_2O .

2.5. Discussion

2.5.1. Effects of Manure Application and Timing on GHG Emissions

2.5.1.1. Timing of Application: Nitrous Oxide. The addition of liquid hog manure to the plots caused an increase in N_2O emissions. This is consistent with the work of others, where animal slurries were surface-applied to grassland (Chadwick et al. 2000; Ellis et al. 1998). Liquid hog manure application may impact N_2O emissions through several microbial processes; biological denitrification, nitrification, and coupled nitrification-denitrification. Denitrification rates can be affected as manure is a source of readily available C and N. Manure application to grassland may also impact nitrification rates by increasing soil NH_4^+ concentrations, producing NO_3^- to be denitrified by denitrifying bacteria (coupled nitrification-denitrification) or NO_2^- to be denitrified by nitrifying bacteria (nitrification as direct source of N_2O) (Wrage 2001). Since soil moisture conditions were highly variable across the study area, it is likely that all three processes could be responsible for the N_2O emissions from the manure-treated grassland. During the transect study, N_2O emissions increased after a heavy rainfall. Soil NO_3^- concentrations and soil moisture were high, conditions favourable for denitrification. In

the anaerobic, saturated areas where N_2O was not produced, it is likely that denitrification was producing dinitrogen gas (N_2) instead. In contrast to this, the dry Spring treatment plot of replicate 2 had the highest emissions of N_2O in 2004. Most of this N_2O came from one sample location on the plot, and this N_2O was produced as NH_4^+ concentrations were dropping, NO_3^- concentrations were climbing, and the soil was dry (9 to 17% gravimetric moisture content). These physical and chemical properties are characteristic of soil conditions where nitrification can occur and be a source of N_2O .

In both years, N_2O was not produced within 24 hours of manure application. This delay of increased N_2O emissions after manure treatment has been observed in the field in several studies (Chadwick et al. 2000; Rochette et al. 2004). A late spring manure application in 2004 occurred during a very wet period, the soil was warming and higher N_2O emissions were produced from the soil only 1-2 days after application. The earlier 2005 spring application occurred when the soil was cool and dry. These conditions would have reduced both nitrification and denitrification rates, thus reducing N_2O emissions. The soil remained dry until rainfall events on May 7th to 9th (DOY 127-129), which corresponded with an increase in N_2O emissions.

The results of this study make it evident that soil N_2O emissions are higher from grassland fertilized with manure than unfertilized grassland. Of greater importance is that N_2O emissions were lower when the grassland in this study was fertilized using a split application of manure in the fall and the spring, rather than when manure was applied solely in the spring. Differences in time of manure application have been shown to influence the emission of N_2O from both grassland and annually cropped soils (Chadwick 1997; Chadwick et al. 2000; Rochette et al. 2004). These studies have

focused on single applications of manure rather than split applications. Chadwick (1997) observed that N₂O emissions from grassland in south-western England were greater when liquid dairy manure was surface applied in the fall than when manure was applied in the spring. This could be due to the lower crop nutrient demand in the fall and winter for grass in England. To the contrary, a study by Rochette et al. (2004) shows that N₂O emissions from an annually cropped maize field in Québec, Canada were higher when liquid hog manure was incorporated in the spring than when manure was incorporated in the fall. The wet and warm conditions that persisted after manure application were said to be responsible for the larger N₂O emissions in the spring rather than the fall.

Fall manure spreading in Manitoba typically occurs late in the year, when soil temperatures are cool. The cool soil temperature decreases the buildup of soil NO₃⁻ and thus decreases the potential loss of N via denitrification and leaching. Although there was some increase in N₂O emissions after fall manure applications to the Split treatments, the large difference in N₂O emissions between the Spring and Split treatments after spring manure application was more important.

The decreased emission of N₂O from the Split treatment plots can be explained several ways. First, little N₂O was emitted from the Split treatment after fall manure application as this manure is applied during a cool period that is getting colder. Cool soil temperatures have a strong influence on nitrification and denitrification activity (Haynes 1986; Aulakh et al. 1992), and there was no apparent increase in CO₂ emission after the fall manure application to the Split plots. A study by Christensen (1983) has shown that variation in N₂O emission from manured grassland was influenced by temperature and that this variation occurred on a diurnal and seasonal basis (with lower temperatures in

fall causing a decrease in N₂O emission). Therefore, a lack of microbial activity (nitrification or denitrification) was a likely factor reducing N₂O emissions in the fall. Another reason for the smaller N₂O emissions from the Split treatment could be that this practice discourages the accumulation of large NO₃⁻ concentrations compared to the large single spring application. Generally, the Spring treatment plots tended to have higher peak nitrate concentrations compared to the Split application plots.

In addition, nitrogen from fall manure application may be lost during spring snowmelt. When snowmelt occurs in Manitoba, the soil is often still frozen, which decreases infiltration of snowmelt, and thus spring is a large runoff period. This is especially true for surface applied manure, where much of the nutrients are stranded near or on the soil surface. However, the land surface at the La Broquerie site is very flat and the soil coarse-textured, thus it is expected that water movement would be vertical (infiltration) rather than lateral (runoff). Nutrient loss after snowmelt may have been a factor in reducing N₂O emissions from Replicate 1 in 2004, as manure was applied to these plots the day before a major snowfall/rainfall event. As the snow melted, nutrients from the manure may have been lost by leaching. A study by Gangbazo et al. (1995) compared runoff NH₄⁺-N losses of spring vs. fall vs. split spring-fall manure application on forages. This study found no significant differences in runoff losses between the different application timings. One reason is that forages provide a continuous cover that reduces the potential for nutrient loss compared to annual cropping systems, thus runoff losses are low when manure is either applied in the spring or in the fall. Although there were no measurements of N loss via leaching, runoff or spring thaw N₂O emissions in this study, one can assume that there were no substantial losses after fall manure

application as the average standing biomass in the Split and Spring manure treatments were similar (8.8 and 8.4 t DM ha⁻¹). In addition, standing forage protein contents in the Split and Spring manure treatments were similar (9.4 and 10.5% (Wilson 2007). A large loss of N during spring would have reduced the yield of the forage on the Split treatment plots.

One period where N₂O emissions were not measured was during the spring thaws of 2004 and 2005, where N₂O emissions can occur when the soil is still very cool. Gas sampling did not occur at this time because spring tends to arrive very suddenly in Manitoba, and it is difficult to time the beginning of a sampling season with the spring thaw. The spring thaw period could potentially be a period of N₂O emission from grassland soil that has been fertilized with manure, as this has been shown to occur in cultivated fields (Chen et al. 1995) and in grassland soil (Müller et al. 2002). If freeze-thaw N₂O emissions were occurring in this study, I would expect them to be larger from the Split treatment than the Spring treatment plots, as N from manure application would be more readily available from the Split treatment at this time. Müller et al. (2002) have found that freezing grassland soil increased the soil concentration of NH₄⁺ and NO₃⁻. This was followed by an increase in N₂O flux once the soil was thawed. The increase in available N during freezing was possibly due to physical disruption of aggregates, exposing exchange sites or to the death of cells.

In this study, there was no evidence of an increase in soil NO₃⁻ in the spring in any of the treatments. Wagner-Riddle et al. (1997) found that grassed soil despite fertilization had low soil NO₃⁻ into the fall and spring compared to annual cropped soil

and concurrently no emissions of N₂O at spring thaw. Thus, freeze-thaw N₂O emissions were likely very low in my study.

2.5.1.2. Manure Application: Methane. Agricultural methane emissions are mostly associated with animal production, and well-aerated land is usually a sink for atmospheric CH₄ (Gregorich et al. 2005). This study showed that a productive perennial grass system having portions saturated due to poor drainage can be a source and not a sink of CH₄.

Liquid hog manure applied to saturated land has the potential to increase methanogenesis, thus creating CH₄ gas. Manure is a source of readily available carbon (C), and has the potential to increase the availability of soil C by increasing root growth. An increase in availability of soil C can cause an increase in CH₄ emissions from a saturated soil environment (Sylvia et al. 1998). Manure application may also cause an increase in soil CH₄ emission by reducing the redox potential of the soil. Manure application to soil can reduce redox potentials by saturating the soil environment, or by increasing heterotrophic organism (Lessard et al. 1997) and plant root O₂ consumption. Reductions of CH₄ consumption and increased concentration of soil CH₄ have been observed after the incorporation of manure into soil (Lessard et al. 1997). Manure application to the plots increased productivity of the manure-treated plots and this translated into greater root-mass in the top 5 cm of soil. The increased productivity of the grassland would also produce larger amounts of readily available C substrates through root turnover and exudates. Thus, an increase in CH₄ emissions from manure-treated grassland may not only be due to the addition of available C with the manure itself, but

more so to the increase in soil available C as a result of increased root exudates and root turnover. This effect would persist long after manure application.

Methane emissions during this study occurred at two different periods in 2004 and in 2005. In 2004, methane emissions occurred on two of the manure-treated plots immediately after manure application. These emissions continued for several days until dropping to near-zero or negative values. Methane emission soon after manure application has been observed by other researchers, although the CH₄ emissions in these studies were much more short-lived (Chadwick et al. 2000; Dittert et al. 2005). Chadwick et al. (2000) found that when dairy and hog slurry was applied to a well-drained grassland in England, 90% of CH₄ was emitted in the first 24 h after application. Dittert et al. (2005) found that CH₄ emissions occurred only for a period of a few hours after cattle slurry application to a coarse-sandy grassland soil in northern Germany. The CH₄ emission from these experiments was most likely a result of volatilization of dissolved CH₄ from the slurry itself. In contrast, the longer-lasting CH₄ emissions shown during this study in the spring of 2004 are most likely due to the fact that manure was spread on saturated ground. Therefore, the increase in CH₄ emissions in the saturated areas observed for days following manure application was most likely caused by an increase in available C due to manure application. The very low or slightly negative CH₄ emissions observed on the Spring treatment plot of Replicate 2 in the spring of 2004 can be attributed to moisture content as the plot was dry (15% gravimetric moisture content vs. 30% in Spring treatment, Replicate 1, average of all data collected), and thus the anaerobic conditions necessary for CH₄ production did not exist. This was the only plot to consistently be a net consumer of CH₄ throughout the two years of the study.

On July 11, 2005 (DOY 192), large emissions of CH₄ occurred on all three hayed plots in Replicate 1 (Control, Split and Spring treatments), and a smaller flux in the Split treatment of Replicate 2. In Replicate 1, the CH₄ emissions were higher in the manure-treated plots. Before the very high CH₄ emission of July 11, heavy rainfall (140 mm over two weeks) brought the water table to the soil surface, with high ambient air temperatures (max. daily temp. of over 30°C for the five preceding days). The very high CH₄ emissions on July 11 (DOY 192) occurred when there was no longer water inside collars at gas sample positions. The water table had risen to the highest level for the 2005 year (Fig. 2.10) on July 2 (DOY 183) and proceeded to drop rapidly by 29 cm by July 11 (DOY 192). Increases in CH₄ emissions on June 16 and June 20 (DOY 167, 171) also corresponded to when the water table was dropping rapidly after a wet period (Fig. 2.10). The gravimetric moisture content of the soil was also lower at this time, thus the soil environment was no longer anaerobic at the time of the high CH₄ emissions.

The episodic burst of CH₄ emission that is coupled with a reduction in the level of the water table has been observed elsewhere in riparian areas of ponds in the prairie pothole region of western Manitoba (Dunmola 2007), in subarctic environments in northern Quebec (Windsor et al. 1992) and in Siberia (Heyer et al. 2002). The high water table and warm weather that preceded the high burst of CH₄ production on July 11 (DOY 192) created an environment where methanogens could be very active. However much of the CH₄ produced during this time would not be released from the soil, as it would be trapped under the water table. The solubility of CH₄ in water is very low (3.5 mL CH₄ in 100 mL H₂O at 17°C), thus the diffusion of CH₄ occurs at a low rate. Instead CH₄ produced below the water table forms gas bubbles under the water table, and once the

water table drops, the hydrostatic pressure exerted on the trapped gas is reduced, and CH₄ can escape via open soil pores (Heyer et al. 2002).

In this study, CH₄ emissions were at times higher from the manure-treated plots than from the Control plots, although this difference was not shown to be significant. The lack of a significant difference is due to very large variation in CH₄ emissions between replicates and years. The transect study demonstrates this well, as CH₄ emissions were shown to occur only in a limited, saturated area. The transect study also provided more evidence that the manure-treated plots produced more CH₄ in 2005. If this study were to be repeated on a smaller scale, in an area that is regularly saturated, it is expected that an increase in CH₄ emissions with manure addition would occur.

2.5.2. Soil Moisture Content Determines Field Variation in GHG Emissions from Manured Grassland Soil

Nitrous oxide and CH₄ emissions can vary over grassland landscapes due to differences in soil moisture content, soil NO₃⁻ and available C (Ambus 1998; Velthof et al. 1996; Velthof et al. 2000 (N₂O); Reiners et al. 1998 (N₂O and CH₄). Soil moisture levels were largely responsible for the large variation in GHG emissions from the plots. In the plot study, it was obvious that the drier areas were largely responsible for the bulk of the N₂O emissions, while the wetter areas were emitting the CH₄ emissions. In addition, CH₄ was often consumed from the atmosphere in the drier areas of the plots, which is consistent with the expectation that agricultural soils are net consumers of CH₄ (Gregorich et al. 2005). After observing this in 2004, the transect study was conducted in 2005 to detail the relation of soil moisture to N₂O and CH₄ emission. The transect study showed that N₂O emissions were largest in the areas with less soil moisture and more soil

NO_3^- , and CH_4 emissions were highest in the wettest (saturated) areas. Manure application had an obvious effect of increasing N_2O emissions in the drier areas, while manure increased CH_4 emissions in the wetter areas. In addition, it appears that CH_4 emissions only occur in areas that are regularly wet throughout the growing seasons. These wetter areas are characterized by the presence of hydrophytic plants (sedges and mosses). These observations are reasonable because methanogens are extremely sensitive to O_2 concentration, so that populations can only become established in areas that are wet most of the time (Topp and Pattey 1997).

The CH_4 emission results in relation to soil moisture were expected and have been observed in the past (Reiners et al. 1998). However, the N_2O emission results in relation to soil moisture were contradictory to several studies where there were larger N_2O emissions at lower slope positions than at higher slope positions (Ambus 1998; Reiners et al. 1998; Velthof et al. 1996; Velthof et al. 2000). A Manitoba study of landscape effects on N_2O and CH_4 emissions by Dunmola (2007) had shown similar results to this study. The study by Dunmola (2007) showed that upslope cultivated areas emitted only N_2O , depressional areas emitted both N_2O and CH_4 and the wettest area of a riparian zone emitted only CH_4 .

There are several reasons why N_2O emissions would be much lower where CH_4 was being produced. First, the saturated soil conditions present at times in the wettest portions of the plots may encourage denitrification, however, most of the N_2O would be reduced to N_2 under these strong reducing conditions (Sylvia et al. 1998). Secondly, the increased available carbon in this very wet area should also encourage the complete reduction of N_2O to N_2 (Arah and Smith 1990). In addition, where it was very wet, there

was little NO_3^- present to be reduced (Fig. 2.16). Low concentrations of NO_3^- in the saturated areas could be caused by a lack of nitrification, leaching of NO_3^- from these soils or rapid denitrification. In contrast, soil conditions in the drier portions of the field were more conducive to nitrification that could produce N_2O but more importantly produce higher soil concentrations of NO_3^- . This NO_3^- could then be denitrified to N_2O after a rainfall (Sylvia et al. 1998). Also, the more aerobic conditions present in the drier areas may increase N_2O emissions by decreasing the reduction of this gas to N_2 .

Agricultural soils in Canada are generally considered to be sources of N_2O and sinks of CH_4 (Gregorich et al. 2005). This study has shown that grassland systems with poor drainage produce N_2O in locations that are not typical of cultivated agricultural systems. While N_2O is generally produced in the wetter, footslope positions of cultivated fields (Van Kessel et al. 1993); in this study, N_2O was produced in the drier portions of the plots. This study has also shown that these pasture and hay systems with poor drainage should be considered as a net source of CH_4 during wet years. The CH_4 consumed during dry periods and in the drier portions of the field is small compared to the emission of CH_4 in saturated areas during wet periods. Manure application increased the N_2O and CH_4 emission from the drier and wetter areas, respectively, although only N_2O increased significantly throughout the plots. Grass-based pasture and hayland in Canada are often situated in areas with poor agricultural capability, as the land is often too wet for annual crops. The N_2O and CH_4 findings of this study are important when considering national greenhouse gas budgets from agricultural systems as grass-based pasture and hay systems situated in poorly drained areas comprise a considerable portion

of agricultural land in Manitoba (south-eastern Manitoba, Interlake) and Canada (southern fringe of Boreal Plains ecozone).

2.6 Summary

In general, surface application of liquid hog manure onto grassland had the effect of increasing N₂O. When the warming potential of both N₂O and CH₄ emissions were estimated, there was also a significant increase in radiative gas flux. Interestingly, the study demonstrated that the Spring treatment of manure produced more N₂O emissions than the Split treatment. The lower emission from the Split treatment could be due to the lower soil temperatures after fall manure application lowering microbial activity to produce N₂O from nitrification and denitrification processes. The lower N₂O emission from the Split treatment may also be due to a loss of manure N over winter, as this N may be more susceptible to loss by runoff or leaching. In addition, this study did not measure emissions during the spring thaw period, which can be a period of high N₂O emission (Chen et al. 1995, Müller et al. 2002).

Soil moisture content was also shown to be a major factor in driving the quantity and type of greenhouse gas emissions from grassland soil. Saturated soil conditions were conducive to CH₄ emission, while drier soil conditions produced N₂O emissions. The discovery of significant CH₄ emissions from this study is important as soils are generally regarded as sinks of CH₄. Pastures and hayland should now be considered as a potential source of CH₄ when soils are saturated. In addition, the observation of a large burst of CH₄ emission after wet conditions had abated is rare.

This study was conducted over two years when there was much more rainfall than normal. Thus, this study needs to be continued in order to measure the effect of manure

application to grassland on greenhouse gas emission during years of normal or less than normal precipitation. Also, in order to determine the total balance of greenhouse gas emissions from manured and non-manured grassland, deep soil samples should be collected and total carbon should be analyzed in order to see if the manured grassland is storing more carbon due to increased root density.

2.7 References

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3 GREENHOUSE GAS EMISSIONS OF ALTERED DUNG AND URINE COMPOSITION FROM BEEF CATTLE GRAZING FORAGE FERTILIZED WITH LIQUID HOG MANURE

3.1 Abstract

Trémorin, Denis G., M.Sc., University of Manitoba, October, 2008. Greenhouse Gas Emissions of Altered Dung and Urine Composition from Beef Cattle Grazing Forage Fertilized with Liquid Hog Manure. Major Professor: Dr. Mario Tenuta.

The objective of this study was to determine the impact of fertilizing forage with liquid hog manure on the composition of dung and urine and resulting emission of greenhouse gases from cattle dung and artificial urine patches. A previous study showed soil moisture to be a major determinant of the emissions of the greenhouse gases nitrous oxide (N₂O) and methane (CH₄) from hayland receiving liquid hog manure. I extended the examination of the role of soil moisture in controlling greenhouse gas emissions in this study to dung and artificial urine patches. Fresh cattle dung was collected from cattle pastures receiving three different liquid hog manure treatments. Manure treatments were no manure (Control); 153 kg per hectare (ha) of available N (two-year average) applied in the spring (Spring); and 149 kg applied per ha (two year average) as half-rate applications in the fall and spring (Split). Artificial cattle urine treatments were developed by converting blood urea data from cattle grazing the three pasture treatments into urine-N concentrations. Four treatments of increasing levels of nitrogen in artificial urine were used (Control, Low, Medium, High). Deionized water was used for the Control treatment. The Low and High treatments corresponded to nitrogen levels of urine expected from cattle from the Control and manure-treated pastures, respectively. The

Medium treatment represented an average urine concentration for the two cattle groups. Two field experiments were conducted. The first field experiment was conducted during two periods of approximately one month each, one period beginning on September 1, 2004 (DOY 245), and one on August 9, 2005 (DOY 221). This experiment examined the effect of manure application treatment and soil moisture conditions on greenhouse gas emissions from cattle dung. The second field experiment was conducted during the second year of the study and examined the effect of the four artificial urine treatments on soil greenhouse gas emissions, as well as the effect of soil moisture on these emissions.

There were manure application effects on N₂O emissions from both patch types. Cattle dung from the Spring treatment pastures produced higher (P≤0.01) N₂O emissions than the other two pasture treatments, with the Control, Split and Spring treatments producing average emissions of 6.3, 30.3 and 81.9 mg N₂O-N m⁻², respectively. All artificial urine treatments produced higher (P≤0.05) N₂O emissions than the Control treatment, and the High treatment produced higher (P≤0.05) N₂O emissions than the Low treatment (average of 4, 170, 550 and 680 mg N₂O-N m⁻² for Control, Low, Medium and High treatment, respectively). Soil moisture conditions had a large effect on N₂O emissions from both dung and urine treatments, with patches in drier areas producing more emissions. Soil moisture conditions had less of an impact on CH₄ emissions from dung patches, as all patches produced CH₄ soon after deposition.

This study demonstrated a negative impact of the application of liquid hog manure to cattle pasture increasing greenhouse gas emissions from both cattle dung and urine patches. The increase in greenhouse gas emissions was most important for cattle urine patches, as there was a strong increase in N₂O emissions from these patches, and

cattle urine patches are a very important source of N₂O emissions from a cattle pasture system. The negative impact of manure application to pasture was more evident on drier soil conditions as N₂O emissions were most enhanced.

3.2 Introduction

It is the nature of grazing mammals to consume large quantities of forage over large areas, and to return large quantities of organic and mineral material as excreta of dung and urine over relatively very small areas. The concentration of labile organic carbon and nutrients in animal dung and urine patches creates a “hot-spot” where microbial activity may be enhanced. Generally, cattle dung and urine patches have nitrogen levels that are too concentrated to be utilized completely by the plants within or around the patch (Haynes and Williams 1993). The excess nitrogen in cattle dung and urine patches is readily lost via several physical, chemical and biological processes. Two of these processes, nitrification and denitrification, can be responsible for the gaseous loss of the greenhouse gas, N₂O, from grassland soils (Müller et al. 1997).

It is difficult to estimate the importance of ruminant grazing on global N₂O emissions. Oenema et al. (1997) reported a range of 0.5 – 3.0 % of excreted N is evolved as N₂O, with an overall mean of 2.0 %, from analysis of several studies conducted mostly in the 1990's. Based on 1996 IPCC data, globally, domesticated animal grazing contributes 1.55 Tg of N₂O-N, which is more than 10% of the global annual flux due to anthropogenic activities (Oenema et al. 1997). There is some evidence that both denitrification and nitrification can contribute to N₂O fluxes from both cattle dung and urine patches (Allen et al. 1996). Dung patches contain large concentrations of readily available N, which can quickly be converted to ammonium (NH₄⁺). In addition, cattle

dung patches contain large amounts of readily available C and the high organic matter content in the patches retains water well, making the patch suitable for denitrification. Nitrification may also occur in the drier areas of the patch more exposed to air and the sun. It is likely that both processes occur in the same dung patch at the same time. Drier areas of the patch are suitable for nitrification, and wetter, inner portions are more suitable for denitrification. Potential losses of N_2O in urine patches are higher than in dung patches because the majority of the N in urine is in the form of urea or other readily degradable forms. In addition, high urine-N concentrations can inhibit complete nitrification and increase soil nitrite (NO_2^-) concentrations through the effect of free ammonia, osmotic stress and pH. Soil NO_2^- is susceptible to gaseous loss in the form of N_2O (Monaghan and Barraclough 1992). These effects may be more severe in coarse-textured soils with high pH, such as the soil in this study (Monaghan and Barraclough 1992).

Cattle dung patches are important sources of CH_4 , although these emissions have been shown to be much less significant than rumen-derived CH_4 emissions (Flessa et al. 1996). Fresh dung patches are sources of CH_4 because they have several characteristics that favour methanogenesis. These characteristics include a readily available carbon source, a high moisture content and high biological oxygen demand. Further, dung patches already contain methanogens from the intestines of cattle. However, weather conditions after the deposition of cattle dung patches can drastically influence the duration and amount of CH_4 emission. Hot, dry conditions decrease the duration and amounts of CH_4 emitted from dung patches, while cool, wet conditions favour longer periods of CH_4 emissions (Holter 1997, Yamulki et al. 1999). In addition, there exists a

strong relationship between the C/N ratio of fresh cattle dung, with more CH₄ being emitted from dung with a lower ratio (Jarvis et al. 1995). Dissolved or entrapped CH₄ may also be released from the dung itself after deposition, as methanogenesis does occur inside the large intestine of ruminants, although little research has investigated this occurrence. Cattle urine patches could influence methanogenesis by the addition of large quantities of water to a small area, and the addition of soluble carbon to the soil (Yamulki et al. 1999). However, methanogens are not ubiquitous throughout soil landscapes, as they are sensitive to aerobic conditions. Thus, enhanced CH₄ emissions due to urine deposition should only occur in soils which are generally saturated throughout the year.

In order to improve productivity, grass-based pastures throughout the world are often fertilized with animal-manures or synthetic fertilizers. The effect of this fertilization on greenhouse gas emissions from grasslands has received a fair amount of attention compared to how this fertilization affects the greenhouse gas emissions from the excreta from the animals grazing this forage. Fertilizing grass-based pastures with manure is not only expected to increase yield, but also increase protein content and N content of the forage (Reid et al. 1966). For a grassland in southeastern Manitoba, spring application of hog manure increased standing biomass from 3.7 to 8.4 t ha⁻¹, while crude protein concentration improved from 7.1% to 10.5% (Wilson 2007). The consumption of grass-hay or legume-hay containing higher N concentrations has been shown to increase daily urine N excretion from beef steers (Archibeque et al. 2001, Basurto-Gutierrez et al. 2003). Anticipated higher concentrations of nutrients in excrement should increase greenhouse gas emissions from dung and urine patches, in particular N₂O, although this

has not been explored despite having important significance to greenhouse gas budgets of grassland systems.

The objective of this study was to examine if a negative impact occurs with fertilization of grass-based forages with liquid hog manure on the emission of N₂O and CH₄ from cattle dung and urine patches. Another objective was to determine the factors (temperature, soil nutrients, etc.) which influence emissions of these greenhouse gases. The influence of soil moisture variability on the quantity and type of greenhouse gas emissions originating from cattle dung and urine patches was of particular interest as this factor was previously found to strongly influence emissions from grassland.

3.3. Materials and Methods

3.3.1. Dung Experiment

Experiments to determine greenhouse gas emissions from cattle dung patches were conducted in 2004 and 2005. These experiments were developed in a manner to determine whether hog manure treatment to grass-based pastures would affect the amount of greenhouse gas emitted from the dung. In 2004, the study began on September 1 (DOY 245) and was completed on September 23 (DOY 267). In 2005, the study period was from August 9 to September 8 (DOY 221-251).

3.3.1.1. Sample Collection and Processing. Cattle dung was collected from pastures (two replicate paddocks of Control, Split and Spring treatments of Grazed pastures) from the same study site detailed in section 2.3.1. Fresh deposited dung was scooped off the ground with a flat shovel, with 10 samples picked from each pasture and placed into individual polyethylene bags. Samples from each paddock were placed into separate

polyethylene bags, as these samples would be placed onto Hayed plots corresponding to the same manure treatment for the Grazed paddocks. Since there was not enough fresh dung to collect on one day of sampling, dung was collected over three days in 2004 (July 8, 16, and 21) (DOY 190, 198, 203), and over two days in 2005 (June 28 and July 14) (DOY 179, 195). On each collection day, samples were collected from all paddocks. The samples were refrigerated at 5°C until preparation for deposition onto Hayed plots. The refrigeration period was between 41 and 54 days in 2004, and between 25 and 41 days in 2005. It is important to note that this long storage time would have altered the nutrient composition of the dung to some degree, as some microbial activity does occur at this temperature. Before the start of each experiment, the dung from each paddock was pooled and mixed together. This mixed sample was then ready for subsampling and placement onto Hayed plots. At this time samples were also sent to a commercial laboratory for analysis (Norwest Laboratories, Winnipeg, MB, now Bodycote Testing Group). One sub-sample sent to the laboratory (Control, Replicate 2) from 2005 was suspected to be contaminated with soil, as it had a very high acid detergent fiber concentration, and a much lower N concentration. This sample was used in the field, as the analyses from the laboratory were received after the experiment had begun. This sample was not used to calculate averages shown in Figs. 3.5, 3.6 and 3.7. The average weight of each dung collection was 1.050 kg fresh weight, and subsamples of this weight of the pooled dung for each paddock were placed into polyethylene bags to create dung paddies in the field. These units of dung were allowed to warm to ambient temperature for approximately 18 hours before they were placed on the field.

3.3.1.2. Plot Layout. The dung experiment was conducted on the hayed plots to avoid disturbance from cattle and variation in emission from previous dung and urine depositions. The static-vented chamber method was used for this experiment to measure greenhouse gas emissions from the dung paddies. Three collars were placed in the plots to measure gases from dung patches. These three collars were placed beside three of the collars from the plot study described in section 2.3.3.1, with two at each end of the plots, and one near the middle. Dung was deposited into a 20.3 cm i.d. white PVC pipe (schedule 40) cut into 5 cm collars, with a bevel end inserted 2 cm into soil and with 3 cm of the collar above the soil surface. This served as a dung paddy for gas sampling. Dung was also deposited into a second collar 1 m beside the collar used for gas sampling and this served for destructive sampling of soil and dung throughout the experiment. A third collar 1 m from the gas sample paddy was positioned and used as a control for gas sampling. These control collars were of similar design as described in section 2.3.3.2.

The number of dung paddies positioned within each plot was three. This allowed one individual per plot to sample dung paddies and control collars for greenhouse gas emissions at four 15 minute intervals. Three individuals sampled from the static-vented chambers, and as such gas collection was complete within 2 hours.

3.3.1.3. Gas Flux Measurement, Soil/Dung Sampling and Analysis. The tops of the static-vented chambers used for gas collection from dung paddies were made with 8 cm sections of 20.3 cm i.d. grey PVC pipe, with a 23 cm diameter circular piece of grey PVC glued to the top of the pipe to form the ceiling of the chamber. These tops were fitted with Nalgene tubing (0.32 cm i.d., 10 cm length) to serve as a vent and a septum stopper for insertion of a syringe for gas collection. The bottom of the top was fitted with a piece

of rubber inner tire tube (Canadian Tire Corporation). This rubber was used as a seal that could be pulled over the crack between the collar and the top during gas collection. The tops were covered in aluminum foil and the rubber was painted white to reduce potential solar heating of the chambers.

Vials for storage of gas were prepared and gas samples collected and stored in the same manner as described in section 2.3.3.2. One exception was that gas samples were taken from the headspace of the chambers at time 0, instead of using atmospheric air to represent the gas in the headspace. This was done because of a concern that fitting of the top would disturb the paddy releasing gas. Gas stored in vials was analyzed for N_2O , CH_4 , and CO_2 and gas emissions were determined as detailed in section 2.3.3.2.

Soil samples were collected before the experiment started, and the fresh dung samples were analyzed to determine initial nutrient concentrations. Dung and soil samples were collected throughout the experiment using an Oakfield tube sampler (diameter = 1.9 cm). Dung paddies were separated into four quarters and three samples were taken from each quarter during each sampling day. Sampling was done in such a manner to minimize disturbance of the paddy (samples were taken at opposite quarters, to minimize disturbance in the next quarter to be sampled). In addition to the dung at the soil surface, 5 cm of soil was also sampled below the patty. The dung and soil were separated into different polyethylene bags and analyzed separately. Dung and soil were processed and analyzed in the same manner as the soil in the studies of section 2.3.3.3, but only 2.5 g of oven-dried dung was used in 25 mL of 0.5 M K_2SO_4 , for a dung/solution ratio of 1:10.

3.3.2. Artificial Cattle Urine Experiment

A field experiment was conducted in 2005 to measure greenhouse gas emissions from artificial cattle urine patches. The experiment compared the greenhouse gas emissions from urine patches excreted by cattle consuming both non-manured and manured forage. The experiment was laid out in a manner so that the effect of a soil moisture gradient in a grass plot on greenhouse gas emissions could be observed. Soil samples were also collected periodically throughout the experiment to observe nutrient and soil moisture changes in the urine patches. This experiment was conducted July 5 to August 31, 2005 (DOY 186-243).

3.3.2.1. Determining Artificial Urine Concentrations. During the summer of 2004, 15 urine samples were collected from steers grazing at the University of Manitoba, La Broquerie research site. The urine was collected while the animals were in holding pens. The urea concentrations of these samples were highly variable from animal to animal. This variability could be caused by many things, including the quantity of water consumed, the types and quantity of forage the animal has been eating, whether the animal is sick or not, etc. After this initial sampling, it was determined that artificial urine would have to be produced in order to represent the urine produced by the animals in the field.

The primary nitrogenous compound found in cattle urine is urea (Bristow et al. 1992) and therefore, it was very important to find a reliable estimate of the concentration of this compound. Studies on dairy cattle have shown that blood urea concentrations can be used to determine the concentration of urea in urine with great accuracy. During this study, blood samples from the grazing steers were collected and analyzed for blood urea

N. A formula by Thornton and Wilson (1972), describes the relationship between the concentration of blood urea N (BUN) and the excretion of urine urea N (UUN). The formula is described as follows:

$$UUN (g/hr) = -0.274 + (0.168 * BUN (mg/100mL))$$

Concentrations of urine urea N were determined by estimating the daily urine output to be 17.5 L per day (Church, 1971). One mistake was made when calculating the concentration of UUN. The units of BUN were given as mmol BUN per L, and when this was converted to mg BUN per L, the molecular weight of urea was used in the calculation, instead of the molecular weight of the nitrogen in the urine. This resulted in 2.14 times the application rate of urea anticipated. Total N concentrations used for this experiment were 4.4, 7.63 and 10.66 g/L, respectively for the *Low* Urine N, *Medium* Urine N and *High* Urine N treatments. These concentrations are comparable to concentrations observed in other studies of dairy cows fed a range of diets, including a grass-based diet. A study by De Boer et al. (2002) on dairy cows found a range of urine N between 4 and 15 g/L, while Bristow et al. (1992) found a higher range for dairy cows between 6.8 and 20.5 g/L. Oenema et al. (1997) report a range of 1-20 g N/L, thus despite the calculation error used in preparation of my artificial urine, the concentrations are well within range of levels reported for dairy cows. However, I do believe that the concentrations used for this experiment are too high for beef cattle grazing grass-based forage, as protein intake by these animals is much lower than dairy cows.

In addition to urea there are other N compounds including allantoin, hippuric acid, creatinine and creatine in the urine of dairy cattle. In terms of N content of urine, the most abundant of these nitrogenous compounds in cattle urine are allantoin (7.3% of

N), hippuric acid (5.8%), creatinine (3.7%), creatine (2.5%) and ammonia (2.8%) (Bristow et al., 1992). Of these compounds, only creatinine was not included in preparing the artificial cattle urine. Creatine was used in the place of creatinine, as these molecules are very similar, both have the same number of nitrogen atoms, and creatinine is formed from creatine when in aqueous solution

The amount of non-urea N in urine was estimated from the relationship developed by de Boer et. (2002) for cattle. The formula describing the relationship is the following:

$$UUN = -1.16 + (0.86 * Total N) \text{ (units g N/L)}$$

Where *UUN* is urine urea N and *Total N* is the total amount of urine N in all forms.

Non urea N was then estimated as:

$$\text{Non-urea N} = \text{Total N} - UUN \text{ (units g N/L)}$$

Table 3.1 illustrates the concentrations of each N component in the artificial cattle urine and the average concentration of urea N in the blood of the cattle at the site. The final concentrations of each component used to make the artificial cattle urine are shown in Table 3.2. The concentration of non-urea N components (except hippuric acid of urine were estimated based on the proportion of these compounds found in the study by Bristow et al. (1992). Hippuric acid was added at a concentration of 4 g per L, which is the saturation point of this compound. Urea, allantoin and creatine were added to satisfy the rest of the N components of the urine. Several salts (NaCl, NH₄Cl, KCl and K₂SO₄) were added, as these cations and anions were found to be prevalent when the actual cattle urine samples were analyzed in 2004. This solution was acidic, and sodium hydroxide was added to bring the solution to a pH of 8. Finally, the electrical conductivity (EC) of the solution was adjusted to 6.8 mS by adding potassium chloride. The pH and EC of the

Table 3.1. Determined cattle blood urea N concentrations and corresponding urine N concentrations.

Treatments¹	Blood urea N² (mmol L⁻¹)	Urine urea N³ (g L⁻¹)	Total urine N (g L⁻¹)	Non-urea urine N (g L⁻¹)
Low	2.17	2.63	4.40	1.78
Med	4.12	5.32	7.53	2.21
High	6.06	8.01	10.66	2.65

1. *Low* corresponds to cattle urine from control pastures, *High* corresponds with cattle urine from manure-fertilized pastures, and *Medium* is an average of the two.
2. Average blood urea N concentration from blood samples taken on a bi-weekly basis during study in 2004.
3. $BUN (mg/100mL) = -0.274 + (0.168 * UUN (g/hr))$ (Thornton and Wilson, 1972)

Table 3.2. Concentration of each nitrogenous compound and salt used to make artificial cattle urine.

	Urea (g L⁻¹)	Allantoin (g L⁻¹)	Hippuric Acid (g L⁻¹)	Creatine monohydrate (g L⁻¹)	NaCl (g L⁻¹)	NH₄Cl (g L⁻¹)	KCl (g L⁻¹)	K₂SO₄ (g L⁻¹)
Low	5.63	2.26	4.00	2.39	0.11	0.11	1.13	0.86
Medium	11.40	2.82	4.00	3.10	0.11	0.11	1.13	0.86
High	17.17	3.38	4.00	3.82	0.11	0.11	1.13	0.86

Note: final pH of urine solutions adjusted to pH 8 with NaOH and electrical conductivity adjusted to 6.8 mS with KCl.

solutions were adjusted as such to match the average results from the fresh urine samples collected in 2004. The average pH found in these urine samples was in agreement with a range found by Richards and Wolton (1976), although the average was on the high end of the range.

Deionized water served as a control treatment. The *Low* Urine N solution corresponds to urine N expected from the control cattle, as these cattle had low blood urea N concentrations. The *High* Urine N solution corresponds to the cattle on the manured pastures, as these cattle had higher blood urea N concentrations. The *Medium* Urine N solution was simply an average N concentration in between the two. The artificial cattle urine solutions were made in deionized water and 10 L batches.

3.3.2.2. Plot Layout. The urine experiment was conducted in an alley between plots 1 and 2 (Fig 2.2). This area was chosen because the alley crossed from a dry, more elevated area to the east, to a wet, lower area to the west. The experiment was laid out to create five replicates with all four treatments, with the replicates placed to cover dry, moderate and wet areas of the alley. Within each replicate, four PVC collars were installed in a line. Each collar received 1 L to its inner area of a particular treatment, either deionized water, *Low*, *Medium* or *High* artificial cattle urine solutions. In addition to the collars used for greenhouse gas measurements, an extra collar for each treatment receiving artificial urine was also placed in four of the replicates to take soil samples throughout the experiment. Only four out of the five replicates were used for soil sampling because there was a lack of artificial urine to treat all five replicates.

3.3.2.3. Gas Flux Measurement, Soil Sampling and Analysis. The static-vented chambers used in this study were exactly the same as the chambers used for the studies in

section 2.3.3.2. The vials were prepared and gas samples were collected and stored in the same manner as in the dung experiment, with gas samples taken from the headspace at time 0. Gas stored in vials was analyzed for N₂O, CH₄, and CO₂ and gas emissions were determined as detailed in section 2.3.3.2.

Soil samples were collected around each collar before the experiment to obtain background available soil inorganic nitrogen values from each replicate and treatment area. Similarly to the dung experiment, artificial urine patches used for soil sampling were separated into four quarters and three samples were taken from each quarter during each sampling day. An Oakfield tube sampler (diameter = 1.9 cm) was used to collect 5 cm soil samples. These soil samples were processed and analyzed as detailed in section 2.3.3.3.

3.3.3. Rainfall, Air Temperature and Soil Temperature

Rainfall was monitored in 2004 and 2005 at the southeast edge of the site with a tipping bucket rain gauge. Air temperature was monitored using a Tidbit temperature data logger (Onset Corporation) suspended 50 cm above the soil surface and shaded by a white, wooden canopy. In addition to the continuous air and soil temperatures collected throughout the experimental periods, soil temperatures beside each chamber and ambient air temperature were measured at each greenhouse gas sampling event. A Traceable Longstem Thermometer (Fisher Scientific Company, Nepean, ON) was used to record these temperatures. Soil temperature was measured at a depth of 2.5 cm and ambient air temperature was taken by holding the thermometer 60 cm above the soil surface in the shade.

3.3.4. Data Analyses

3.3.4.1. Dung Experiment. A general analysis of variance (ANOVA) was used to determine if there were significant effects of manure treatment on the cumulative emissions of N₂O and CH₄ from dung patches, as well as the combined greenhouse warming potential (CO₂ equivalents) of these two gases. Linear interpolation (Pennock et al., 2006) was used to fill gaps in data where sampling did not occur to calculate cumulative gas emissions. In order to calculate cumulative gas emissions from cattle dung, the gas emissions of the control chamber was subtracted from the gas emissions of its corresponding dung patch. The cumulative dung gas emissions had to be log transformed to improve the normal distribution of the data set. For this test, manure treatment was specified as the fixed effect, while replicate-year and the treatment*replicate-year interactions were considered as the random effects of the model. Both PROC GLM (SAS Institute Inc. 2000) and PROC MIXED were used to perform ANOVA on the data set, depending on whether the variances of the standard deviations were distributed homogeneously or not across the treatments. LSMEANS were then calculated to test for significant differences between the treatments. Where significant treatment effects were observed, Tukey's test determined the minimum significant difference between manure treatments at $\alpha = 0.05$.

PROC CORR (SAS Institute Inc. 2000) was used to perform Spearman Rank correlation analysis between several dependent (N₂O, CH₄, CO₂ and equivalent CO₂ fluxes) and independent (dung NH₄⁺, dung NO₃⁻, soil temperature and dung moisture) variables collected. Correlation analysis was only performed on sampling days where

both dung and gas samples were collected. Scatterplot graphs of dependent/independent variable combinations are presented where obvious relationships were present.

3.3.4.2. Urine Experiment. A typical ANOVA was not suitable for this data set, as variation in gas emissions between replicates were too high, due to the large differences in soil moisture between the blocks. Since the differences in gas emission were obviously related to soil moisture, an analysis of covariance was used to determine if there were significant differences between the four treatments on the cumulative emissions of N₂O, CH₄ and CO₂. In order to calculate cumulative gas emissions, linear interpolation (Pennock et al., 2006) was used to fill gaps in data where gas sampling did not occur. The cumulative urine gas emissions of N₂O and CH₄ had to be log transformed to improve the normal distribution of the data set; this was not the case for the CO₂ data. For this test, manure treatment was specified as the fixed effect, while Replicate and soil moisture were considered the random effects of the model. Soil moisture content was included in the analysis as the covariable influencing greenhouse gas emission; the analysis of covariance adjusted treatment means in order to perform a better estimate of manure treatment effect. PROC GLM (SAS Institute Inc. 2000) was used to perform the analysis of covariance. Finally, a Tukey's test of the treatment means determined the minimum significant difference between manure treatments at $\alpha = 0.05$.

Correlation analysis was also performed on the data where both soil and gas samples were collected. PROC CORR was used to perform Spearman Rank Correlation analysis between measured variables. Two scatterplot graphs are presented showing the relationship between N₂O emission and soil NO₃⁻ and NO₂⁻ concentrations.

3.4. Results

3.4.1. Dung Experiment

3.4.1.1. Fresh Dung Characteristics. Characteristics of the dung used in the Dung Experiment were affected by manure treatment of the forage. For 2004, Spring manure treatment resulted in dung with greater Total N, Organic N, and lower acid detergent fiber (ADF) and neutral detergent fiber (NDF) compared to the other treatments (Table 3.3). In 2005, dung from the Spring treatment was not different in measured parameters compared to the Split treatment. The characteristics of the two manure treatments of the dung used in 2005 seem to have had greater Total N, Organic N and ammonium compared to dung taken from Replicate 1 of the Control treatment.

Many of the preceding treatment effects on the physical and chemical parameters of the cattle dung were visually apparent when the cattle dung was collected. In 2004 and 2005, the cattle dung that was collected from pastures treated with hog manure were often darker green than the cattle dung from the Control pastures. The steers on these manure treated pastures would also produce dung that had less coarse material in it. These two effects were most evident in the dung from the Spring application pastures. The darker green colour was indicative of more chlorophyll and thus more N in the dung. This is also a reflection of greener forage on manured than Control paddocks. The finer textured manure would be more highly digestible for microorganisms, due to the greater surface area of this material, and the lower concentration of slowly degradable substrates, such as acid detergent fiber (lignins).

Table 3.3. Physical parameters and nitrogen concentration of fresh cattle dung collected from pasture. All nitrogen and fiber measurements are on a dry basis.

2004	Moisture (%)	pH	EC¹ (dS m⁻¹)	Total N (g kg⁻¹)	Organic N (g kg⁻¹)	Ammonium-N (mg kg⁻¹)	Nitrate-N (mg kg⁻¹)	ADF² (%)	NDF³ (%)
Control Rep 1	85.0	8.3	24.0	20.8	20.1	770	13	39.6	57.7
Control Rep 2	85.6	8.4	22.1	18.6	17.9	770	<5	39.6	56.7
Split Rep 1	86.3	8.5	29.1	19.9	18.6	1260	10	42.3	53.3
Split Rep 2	86.5	8.3	29.3	18.3	16.4	1860	16	42.2	57.3
Full Rep 1	87.9	8.5	33.6	27.8	26.2	1610	7	33.3	50.9
Full Rep 2	86.6	8.6	32.8	28.0	25.7	2260	<5	37.6	38.1

2005	Moisture (%)	pH	EC¹ (dS m⁻¹)	Total N (g kg⁻¹)	Organic N (g kg⁻¹)	Ammonium-N (mg kg⁻¹)	Nitrate-N (mg kg⁻¹)	ADF² (%)	NDF³ (%)
Control Rep 1	87.6	8.3	34.1	18.7	17.0	1630	15	41.0	59.8
Control Rep 2*	75.1	8.6	16.4	8.7	7.9	800	<4	68.2	28.8
Split Rep 1	88.3	8.8	35.6	22.4	19.7	2760	14	39.5	59.6
Split Rep 2	87.6	8.7	31.6	21.8	19.9	1950	11	39.6	59.0
Full Rep 1	88.6	8.5	35.5	22.3	20.8	1510	4	39.0	58.4
Full Rep 2	86.1	8.1	34.8	22.3	21.0	1280	14	41.6	55.7

* sample contaminated with soil (sample not used to calculate averages for Figs. 3.5, 3.6 and 3.7).

¹ electrical conductivity

² acid detergent fiber

³ neutral detergent fiber

3.4.1.2. Gas Emissions in 2004 and 2005.

3.4.1.2.1. Nitrous Oxide. In general, N₂O emissions were higher from the dung patches than from the surrounding grassland. Nitrous oxide emissions were generally highest from dung collected from steers grazing the Spring treatment, followed by dung from the Split treatment (Fig. 3.1). However, in 2005, the dung from the Split treatment produced N₂O emissions similar to the dung from the Control pastures. The dung from the Control pastures produced the least amount of N₂O. The maximum measured N₂O emission from any dung patch from the manure-treated pastures was over six times larger than that from any dung patch from the Control pastures. When N₂O emissions occurred from the dung patches, there was high variability between replicates and within plots. For example, on September 16, 2004 (DOY 168), the dung in the Spring plot of Replicate 1 emitted an average of 173 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ with a standard error of the mean of 135 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. All the N₂O from this plot was emitted from two chambers. In comparison, on September 2, 2004 (DOY 246), this same plot emitted an average of 2.86 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ with a standard error of the mean of 8.16 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$.

After deposition of dung, there was a delay of two to five days in the emissions of N₂O gas. Emission of N₂O gas from the dung patches was probably delayed because of the initial low concentration of nitrate within the dung. In 2004, N₂O emissions from dung had largely dropped 21 days after dung deposition, with the Spring plot in Replicate 1 still producing some emissions. This was a wet period of time with water in some of the chambers, although the two chambers that were emitting N₂O were in drier areas of the plot. In 2005, N₂O emissions from the deposited dung had dropped by 29 days after dung deposition. On August 25, 2005 (DOY 237), there was an increase in N₂O emission

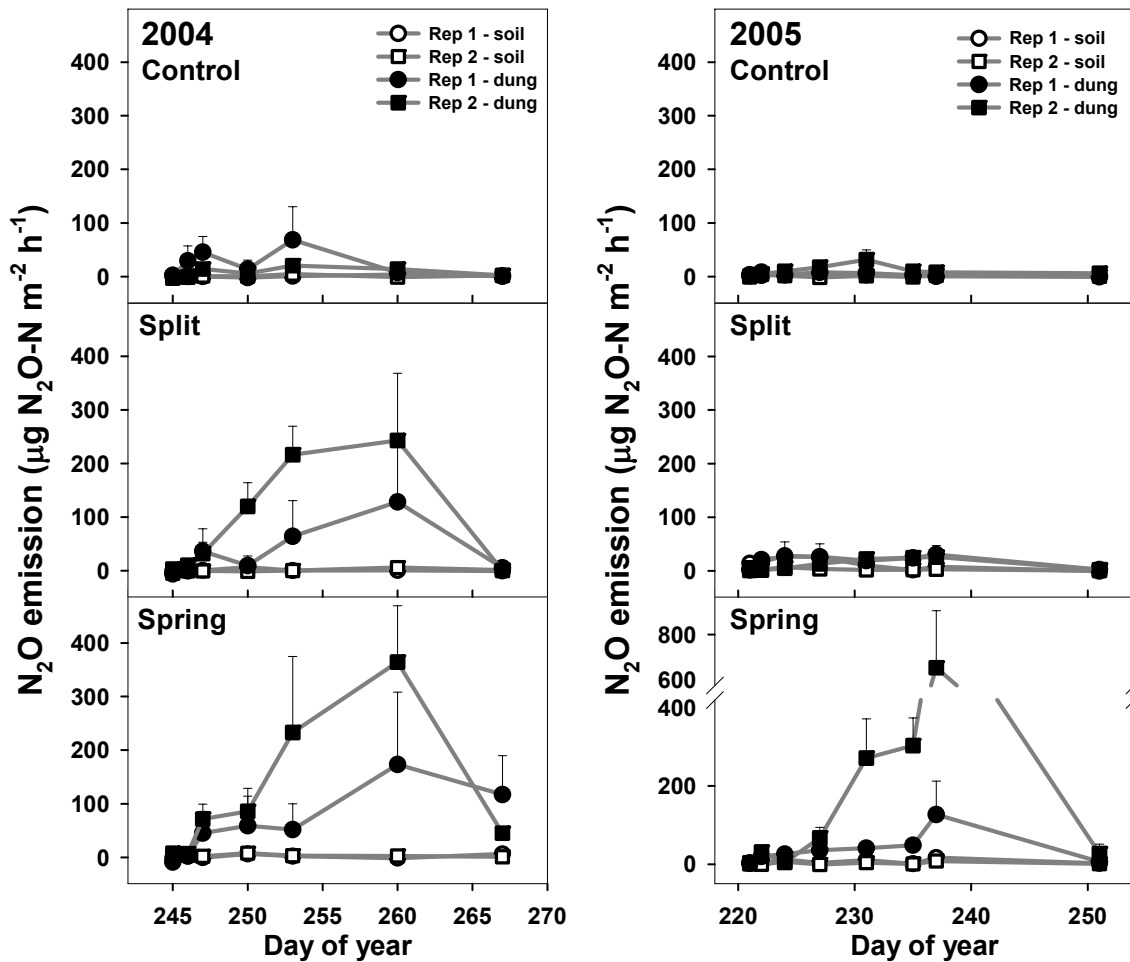


Figure 3.1. Nitrous oxide emission from the Dung Experiment during sampling periods of 2004 and 2005. Values represent average of 3 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1, square points represent Replicate 2 results. Hollow points represent emissions from the untreated soil surface; filled points represent emissions from cattle dung patches. Note y-axis scale break for N₂O emission for Spring treatment.

from dung originating from the Spring manure treatment. This increase coincided with an increase of 3.1 mg NO₃⁻-N per kg of dry dung, and with 7.3 mm of rainfall the previous night.

3.4.1.2.2. Methane. Methane emissions were of short duration, released for one week following deposition of dung (Fig. 3.2). The control treatment (without dung) did not emit CH₄. In 2004, the duration of CH₄ emissions was less than in 2005. Generally peak CH₄ emissions occurred soon after deposition with rates being higher in 2005 than 2004. Emissions occurred for 1 week compared to 3 days for 2005 and 2004, respectively (Fig. 3.2).

3.4.1.2.3. Carbon Dioxide. Similar to N₂O, CO₂ emissions were higher from dung originating from manure-treated pastures than that originating from Control pastures (Fig. 3.3). Carbon dioxide emissions from dung patches were generally higher in 2005 than 2004 and likely due to warmer temperatures during the study period of 2005 than 2004. CO₂ emissions increased briefly after deposition of dung. CO₂ emissions also increased after rainfall events, such as for August 25, 2005 (DOY 237). This coincided with a sharp increase in N₂O emission for dung from Spring pastures. By the end of the study periods, CO₂ emissions from dung patches approached “background” levels from the soil surface.

3.4.1.3. Treatment Effect on Cumulative Dung Gas Emissions. Nitrous oxide emission from dung patches varied greatly between replicates and years. However, in 2005, there was a notable lack of N₂O emission from cattle dung of the Split treatment (Table 3.4). Over both study years, the Spring treatment produced significantly (P≤0.01) higher cumulative N₂O emissions than other dung treatments.

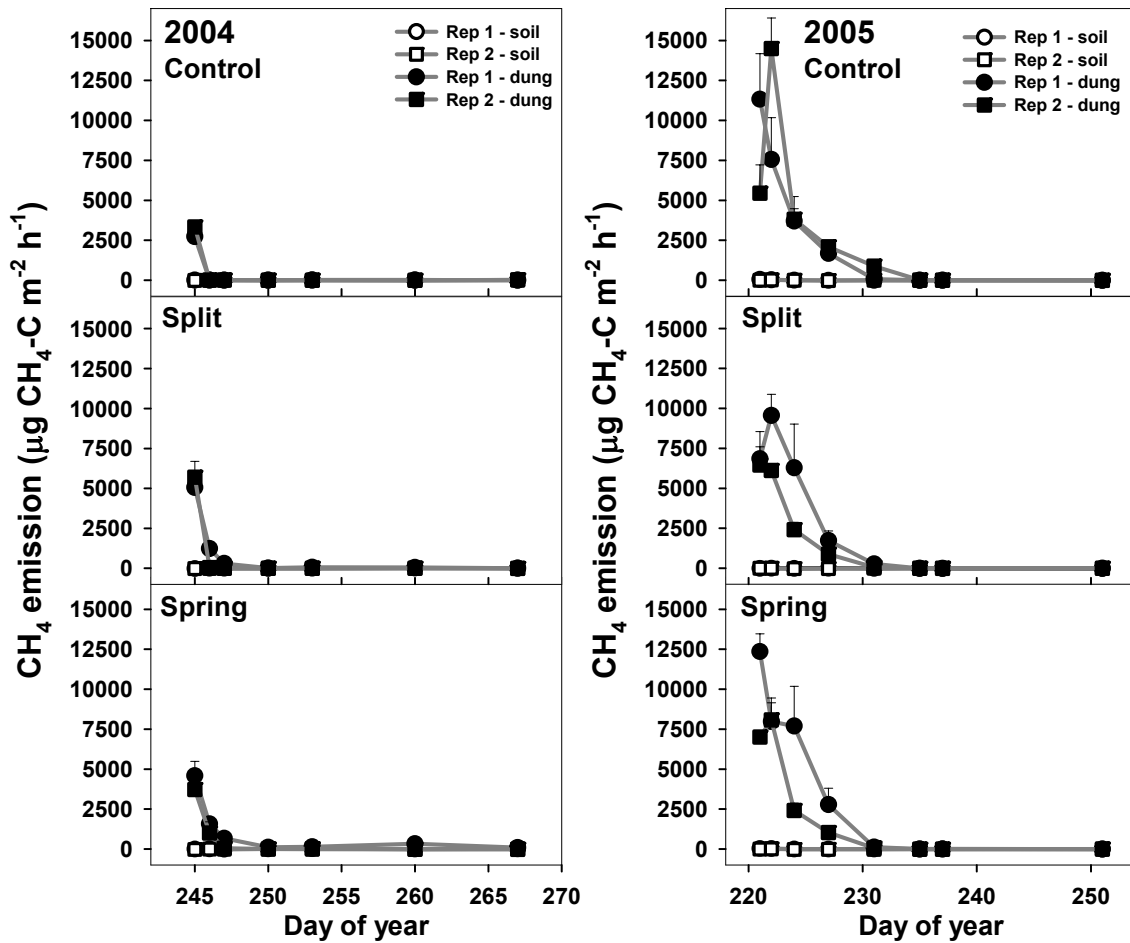


Figure 3.2. Methane emission from the Dung Experiment during sampling periods of 2004 and 2005. Values represent average of 3 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1, square points represent Replicate 2 results. Hollow points represent emissions from the untreated soil surface; filled points represent emissions from cattle dung patches.

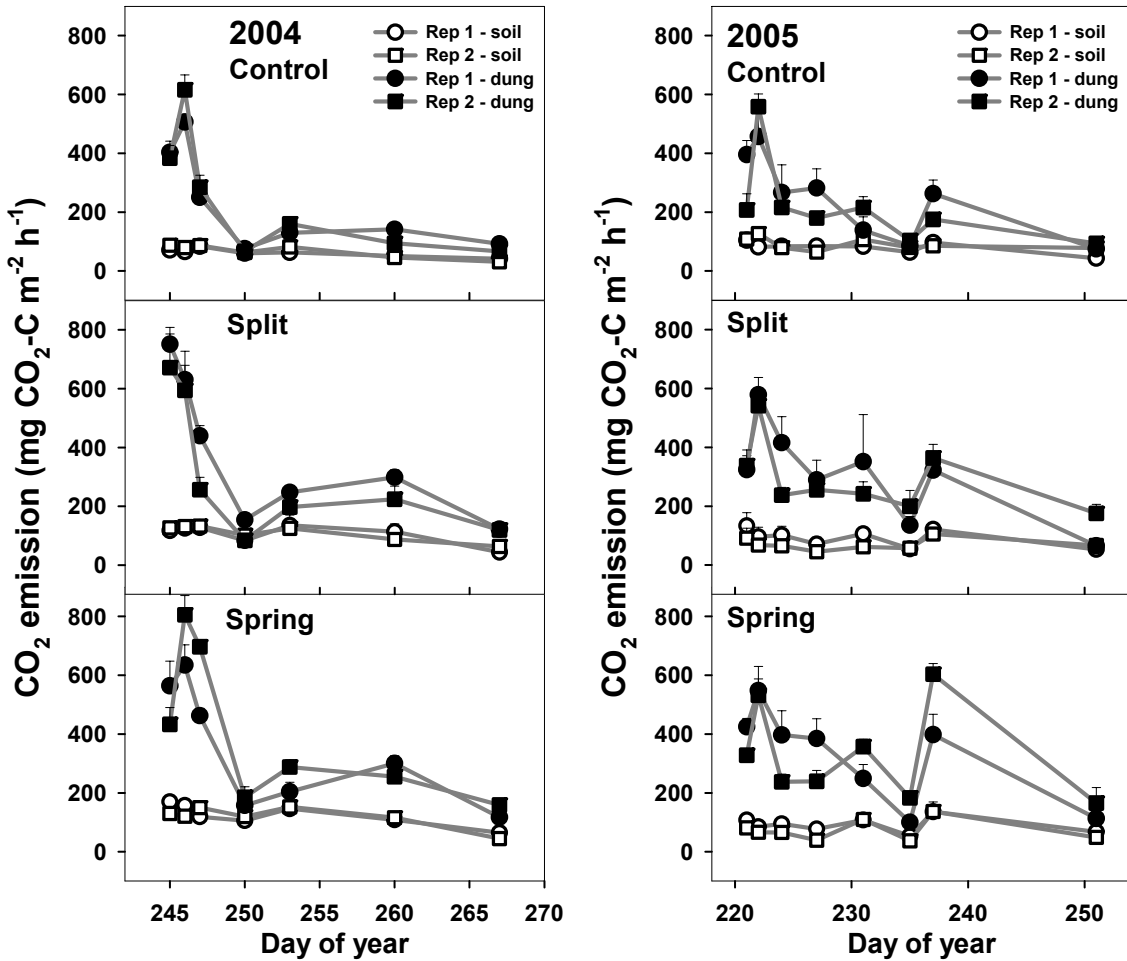


Figure 3.3. Carbon dioxide emission from the Dung Experiment during sampling periods of 2004 and 2005. Values represent average of 3 sample periods, and error bars represent +1 standard error of the mean. Round points represent Replicate 1, square points represent Replicate 2 results. Hollow points represent emissions from the untreated soil surface; filled points represent emissions from cattle dung patches.

Table 3.4. Cumulative greenhouse gas emissions from cattle dung patches in 2004 and 2005. Mean values followed by the same letter are not significantly different, based on log transformed data (Tukey). Values for each replicate (Rep 1 and Rep 2) represent means of 3 sample positions. Values for average and standard deviation represent means of 6 sample positions (3 positions for each replicate over the two years of the study).

N₂O						
	2004		2005		Average	St. Dev.
	Rep 1	Rep 2	Rep 1	Rep 2		
	mg N₂O-N m⁻²				P≤0.01	
Control	12.69	5.38	0.45	6.84	6.34 b	10.60
Split	31.46	76.45	5.65	7.47	30.26 b	40.50
Spring	51.79	101.85	26.30	147.83	81.94 a	68.66

CH₄						
	2004		2005		Average	St. Dev.
	Rep 1	Rep 2	Rep 1	Rep 2		
	mg CH₄-C m⁻²					
Control	63.2	82.9	914.5	1146.0	551.7	561.7
Split	179.6	141.2	1059.5	607.5	497.0	451.7
Spring	266.4	120.6	1289.3	704.9	595.3	510.5

CO₂						
	2004		2005		Average	St. Dev.
	Rep 1	Rep 2	Rep 1	Rep 2		
	g CO₂-C m⁻²				P≤0.01	
Control	55.8	50.7	80.0	61.5	62.0 b	20.7
Split	99.3	65.9	117.8	119.7	100.7 a	33.5
Spring	85.2	105.3	124.5	155.5	117.6 a	34.6

CO₂ equivalents (combined N₂O and CH₄ emissions)						
	2004		2005		Average¹	St. Dev.
	Rep 1	Rep 2	Rep 1	Rep 2		
	g CO₂ m⁻²				P≤0.10	
Control	7.95	4.95	25.87	35.47	18.56 c	15.33
Split	20.36	41.19	32.47	20.68	28.68 b	16.16
Spring	32.69	52.99	48.97	91.77	56.61 a	32.77

¹ P≤0.10, with difference between Control and Spring: P≤0.01, difference between Control and Split: P≤0.05, difference between Spring and Split: P≤0.10.

Methane emissions did not differ between treatments. Generally, there were much higher emissions in 2005 than 2004 for all treatments. Dung from manure treatments produced significantly ($P \leq 0.01$) higher cumulative CO₂ emissions than the Control (Table 3.4). Overall, cumulative CO₂ emissions from all treatments were higher in 2005 than 2004. Further, the dung on the driest plot of the study (Rep 2, Spring application) had the highest cumulative CO₂ emissions, as it did for N₂O emissions.

Cumulative CO₂ equivalent emissions (N₂O plus CH₄) were calculated by combining the dung N₂O and CH₄ emissions. Overall, the 2005 study year produced higher CO₂ equivalents than in 2004. Of the greenhouse gases emitted from dung, N₂O was clearly the most important contributor of total emissions of CO₂ equivalents in 2004 whereas CH₄ was in 2005. Generally, there was an increase in CO₂ equivalents in order of Control, Split to Spring treatments ($P \leq 0.10$).

3.4.1.4. Physical Conditions.

3.4.1.4.1. Rainfall, Air Temperature and Soil Temperature. The study periods were different in terms of rainfall and air temperature (Fig. 3.4). 2004 was generally wetter, with rainfall before and throughout the study (125 mm from August 24 to September 23) (DOY 237-267), resulting in standing water on the west portion of Replicate 1, submerging the dung patches. In contrast, rainfall was much less in 2005 with no appearance of standing water (36.3 mm from August 2 to September 8) (DOY 214-251). The air temperature was generally higher during the 2005 study period (average 16.4°C), although there were some hot days during the 2004 study period (average 15.1°C). Soil temperature at 5 cm depth followed the same pattern as the air temperature for both years (data not shown).

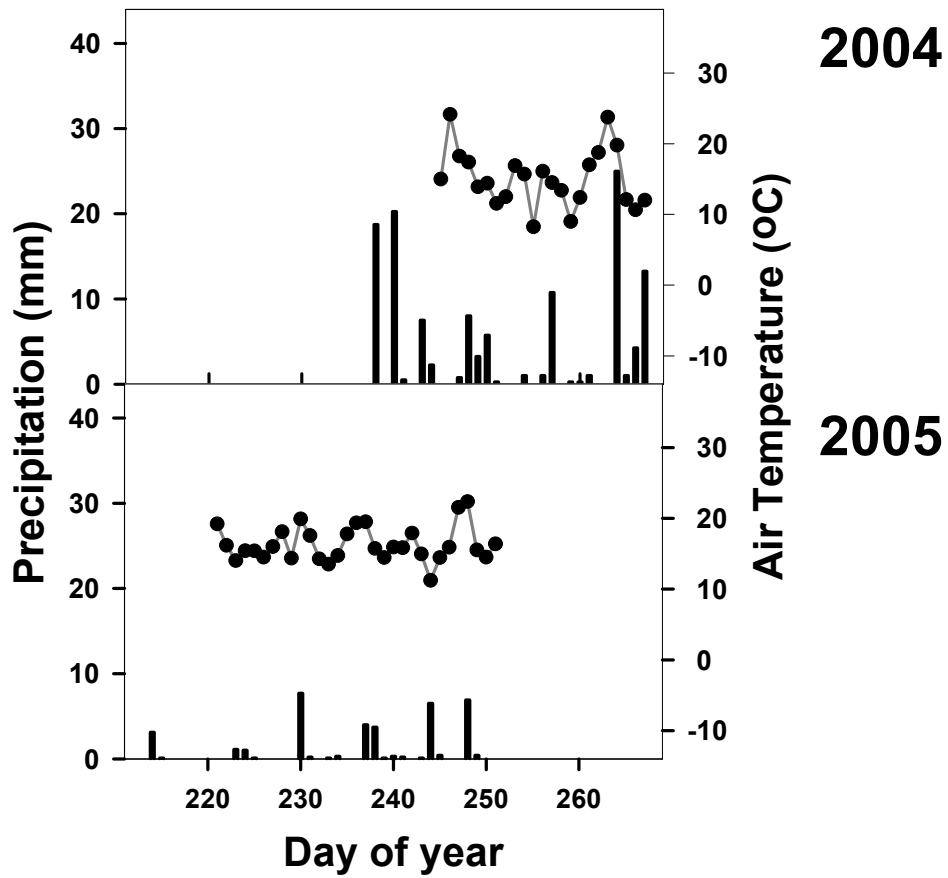


Figure 3.4. Daily total precipitation and average air temperature during the Dung Experiments of 2004 and 2005. In order to show the occurrence of precipitation prior to the experiments, precipitation data from one week before the start of the experiments is included.

3.4.1.4.2. Dung Conditions. The gravimetric moisture content of dung was generally much higher than that of the soil underneath the dung (Fig. 3.5). In 2004, dung in Replicate 1 increased in moisture content after deposition, while in Replicate 2, all dung treatments except for the Control decreased in moisture content. After the first sampling, the moisture content of the dung in the two replicates approached each other. It is important to note that in 2004, dung in Replicate 1 was often placed onto saturated soil conditions. In 2005, the moisture content of the dung decreased initially after deposition, but then it increased after a rainfall. Conditions were drier in 2005 than in 2004, though dung moisture contents became higher in 2005. This is most likely due to dung sampling occurring after some small rainfall events in 2005. It is important to note the high insect activity in the dung patches in 2004 compared to 2005. This may have acted to aerate and dry the patches more effectively in 2004.

Generally, ammonium (NH_4^+) concentrations by weight were much higher in the dung than in the soil (Fig. 3.6). Initial dung concentrations of NH_4^+ decreased a minimum of 7.5 times five days after deposition. This sharp decrease in NH_4^+ concentration can be due to volatilization, nitrification, leaching and uptake by microbial biomass. In 2004, the NH_4^+ concentration tended to increase after five days, and then slightly decreased after 15 days. This did not occur in 2005.

Compared to the dung NH_4^+ concentration, NO_3^- concentrations by weight in the cattle dung were similar to that of soil (Fig. 3.7), even in fresh samples before deposition. This was especially true in 2004, when NO_3^- concentrations in the soil were even higher than the dung on several occasions. In contrast to dung NH_4^+ concentrations, the initial fresh dung samples had similar levels of NO_3^- to the field sampled dung.

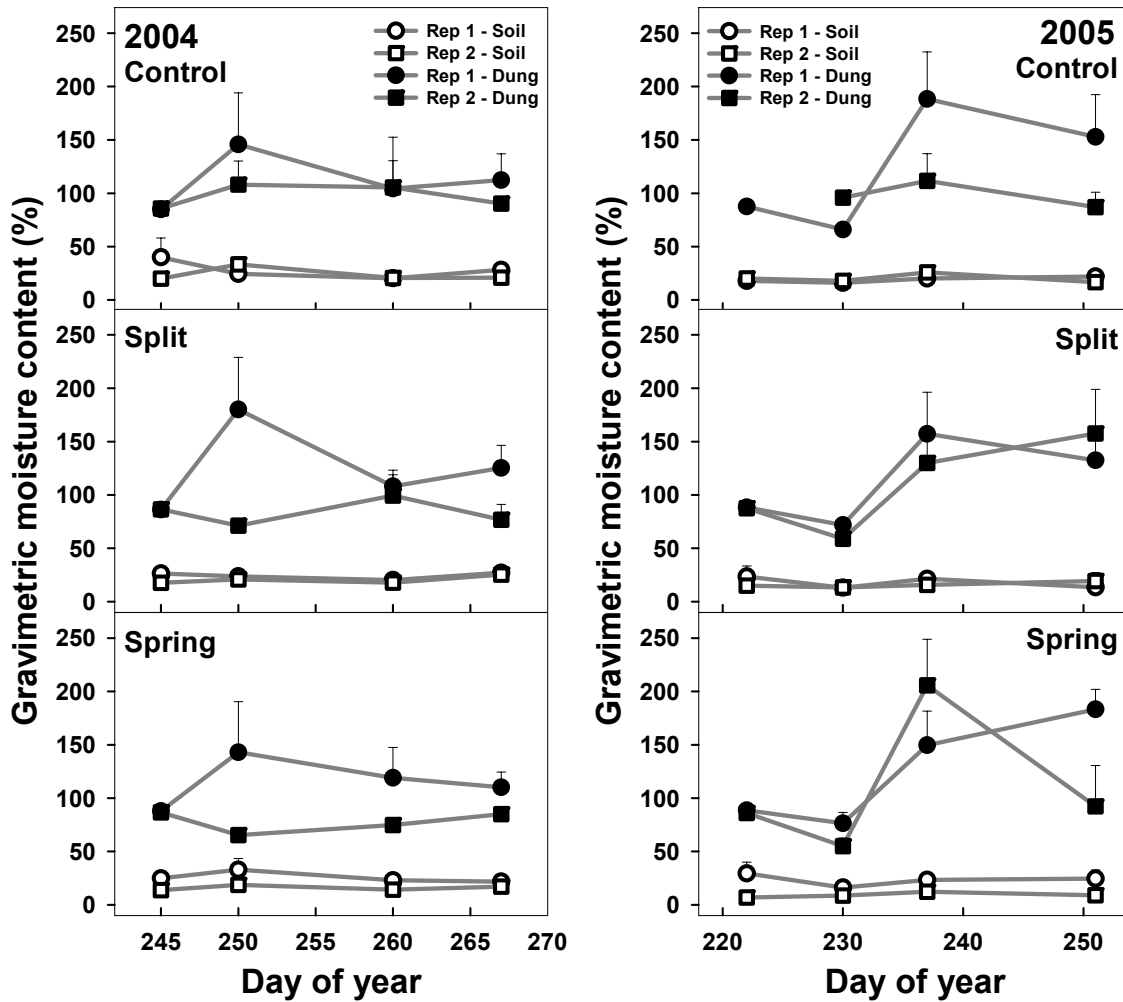


Figure 3.5. Dung and soil gravimetric moisture contents from the Dung Experiment during sampling periods of 2004 and 2005. Values represent average of 3 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1, square points represent Replicate 2 results. Hollow points represent emissions from the untreated soil surface; filled points represent emissions from cattle dung patches. Dung results shown for initial day of sampling represent fresh dung before deposition. Note the missing data point for Control, Dung, Replicate 2, this sample was contaminated.

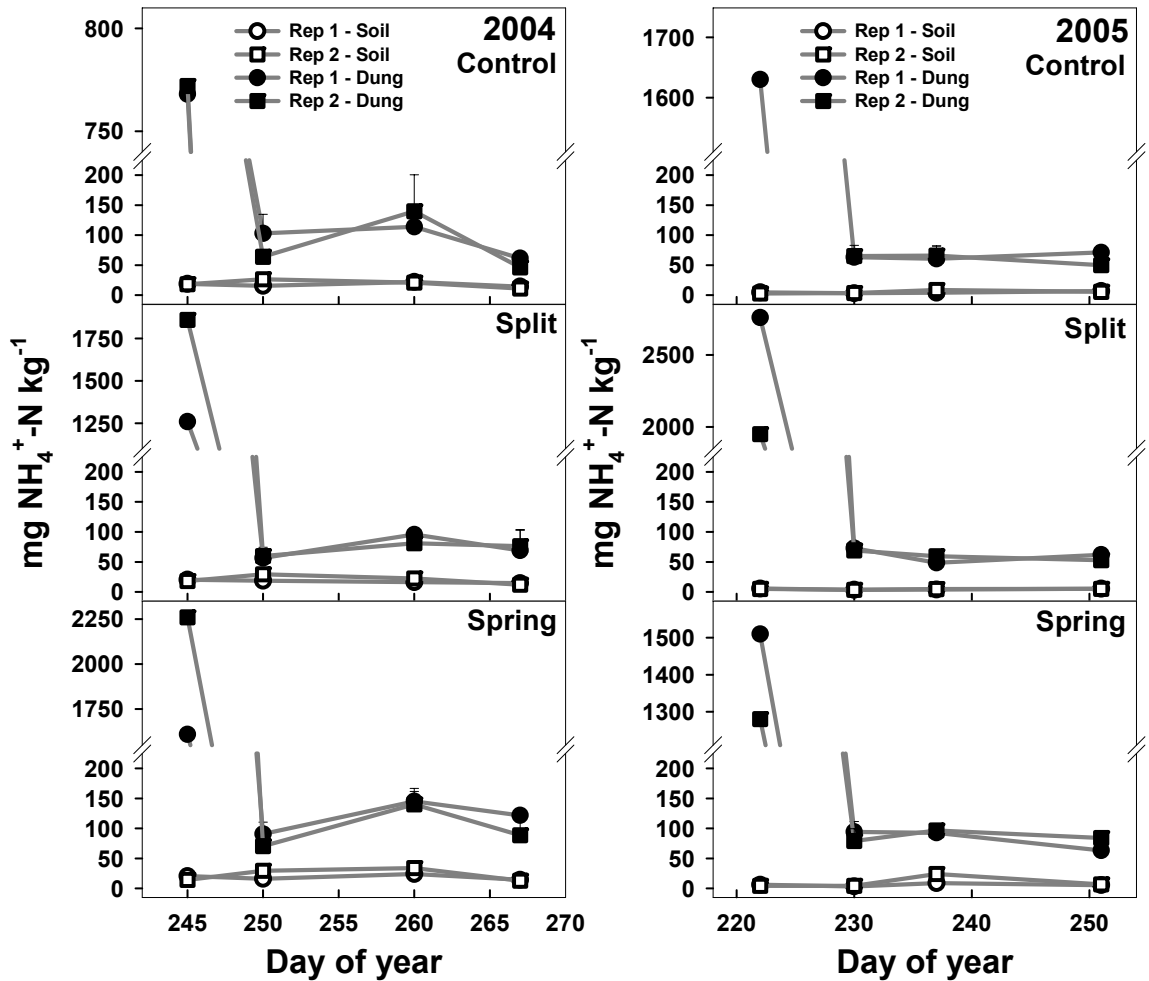


Figure 3.6. Dung and soil ammonium concentrations (dried basis) from the Dung Experiment during sampling periods of 2004 and 2005. Values represent average of 3 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1, square points represent Replicate 2 results. Hollow points represent emissions from the untreated soil surface; filled points represent emissions from cattle dung patches. Note the differences in scale for the Y axis. Dung results shown for initial day of sampling represent fresh dung before deposition. Note the missing data point for Control, Dung, Replicate 2, this sample was contaminated.

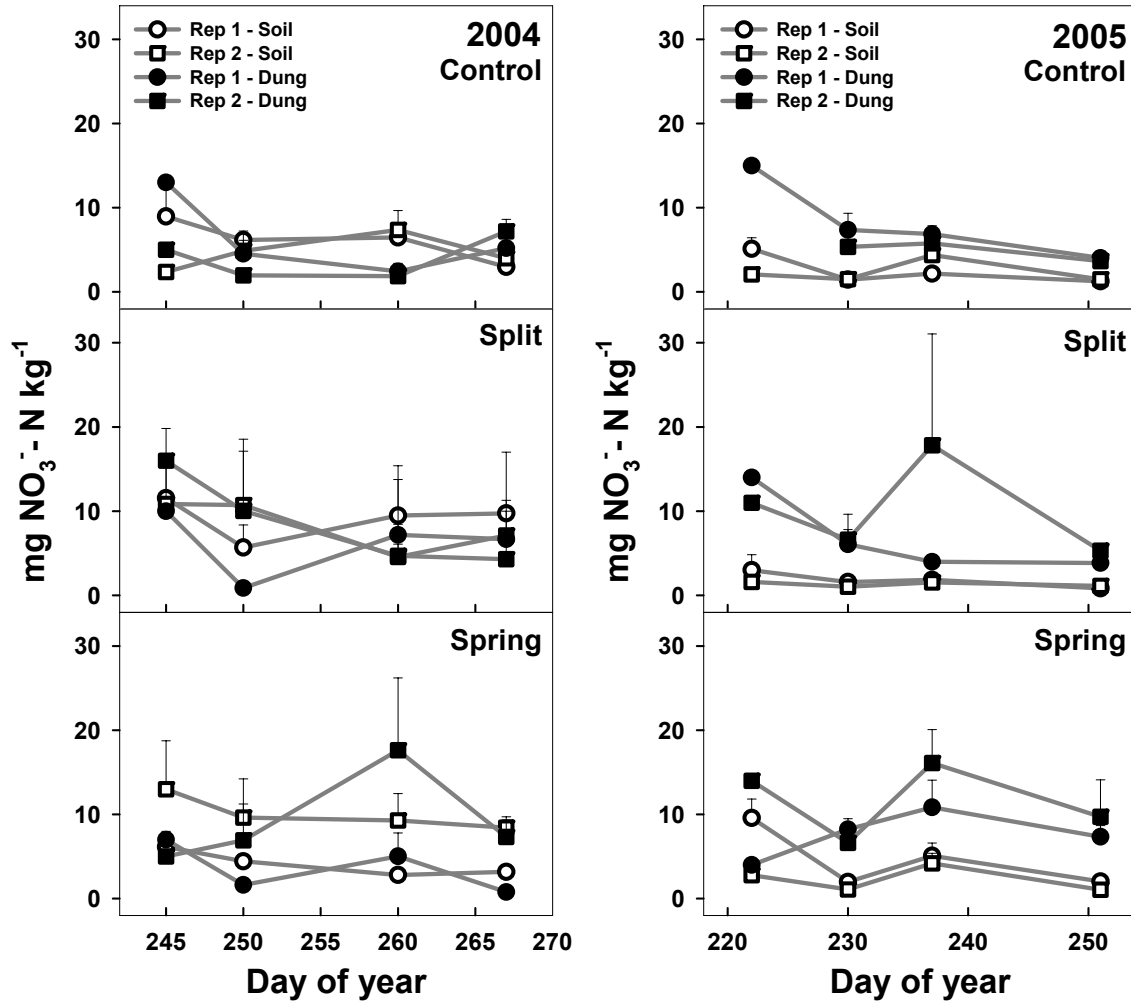


Figure 3.7. Dung and soil nitrate concentrations (dried basis) from the Dung Experiment during sampling periods of 2004 and 2005. Values represent average of 3 sample positions, and error bars represent +1 standard error of the mean. Round points represent Replicate 1, square points represent Replicate 2 results. Hollow points represent emissions from the untreated soil surface; filled points represent emissions from cattle dung patches. Dung results shown for initial day of sampling represent fresh dung before deposition. Note the missing data point for Control, Dung, Replicate 2, this sample was contaminated.

3.4.1.5. Correlation of Measured Parameters to Gas Emissions.

3.4.1.5.1. Correlation Matrix. Similarly to Chapter 2, many of the variables in the dung study were not strongly correlated, and had low correlation coefficients (r-value) (Table 3.5). However, the power of the correlations was often very good, with the probability level approaching zero when correlation coefficients were higher than 0.35. Nitrous oxide emissions from the dung patches were positively correlated with CO₂ emissions and dung NO₃⁻. A weaker positive correlation existed between N₂O emissions and soil NH₄⁺, while negative correlations were found between dung N₂O emissions and the moisture content of dung and soil. There were no strong correlations between CH₄ emissions and any of the parameters, as the bulk of the emissions occurred soon after dung deposition, and no soil or dung samples were taken during this period. In addition to being associated with N₂O emission, dung CO₂ emissions were positively correlated with dung NH₄⁺ and NO₃⁻. There was a particularly strong correlation between dung CO₂ emissions and soil temperature. There were many other correlations between the non-gaseous variables measured in this study. Particularly strong correlations were found between soil NH₄⁺, soil NO₃⁻ and soil moisture.

3.4.1.5.2. Scatterplot. Simple scatterplot graphs showing the relationship of variables on N₂O and CH₄ emissions are given in Figure 3.8. The range of dung gravimetric moisture content that produced high emissions of N₂O was much larger than that seen for hog manure applied to grassland in Chapter 2 (Fig. 3.8, *a*). This range was about between 50 and 150%, and there were outliers at higher moisture contents. The highest CH₄ emissions were produced from a more narrow range, most being produced 80-90% moisture content (Fig. 3.8, *b*). Nitrous oxide fluxes occurred at a wide of range of dung

Table 3.5. Spearman Correlation Coefficients and probability level for the Dung Experiment (both replicates and both years; n=108). Values in bold show strong correlation between two variables. Note that data from the first day of sampling was not included, as high CH₄ and CO₂ emissions on that day would skew the results shown.

	N ₂ O	CH ₄	CO ₂	dung NH ₄ ⁺	dung NO ₃ ⁻	dung m.c.	soil NH ₄ ⁺	soil NO ₃ ⁻	soil m.c.	soil temp.
N ₂ O	1.00000									
CH ₄	0.08649 0.3734	1.00000								
CO ₂	0.40826 ≤.0001	-0.12036 0.2147	1.00000							
dung NH ₄ ⁺	0.18971 0.0492	0.09769 0.3145	0.20741 0.0313	1.00000						
dung NO ₃ ⁻	0.32204 0.0007	-0.16373 0.0904	0.26988 0.0047	-0.04638 0.6336	1.00000					
dung m.c.	-0.29386 0.0020	-0.0315 0.7462	0.06310 0.5165	-0.03536 0.7164	-0.15984 0.0984	1.00000				
soil NH ₄ ⁺	0.21077 0.0286	-0.18822 0.0511	-0.18140 0.0603	0.36402 ≤.0001	-0.09261 0.3405	0.02460 0.8005	1.00000			
soil NO ₃ ⁻	0.18232 0.0590	-0.09085 0.3497	-0.05692 0.5584	0.27163 0.0045	0.05579 0.5663	-0.01353 0.8894	0.76198 ≤.0001	1.00000		
soil m.c.	-0.3751 ≤.0001	0.16656 0.0849	-0.16571 0.0865	-0.00567 0.9536	-0.35802 ≤.0001	0.46672 ≤.0001	0.25834 0.0069	0.26632 0.0053	1.00000	
soil temp.	0.17781 0.0656	0.07864 0.4185	0.44775 ≤.0001	-0.06183 0.5249	0.35945 ≤.0001	0.12971 0.1809	-0.53781 ≤.0001	-0.55383 ≤.0001	-0.36001 ≤.0001	1.00000

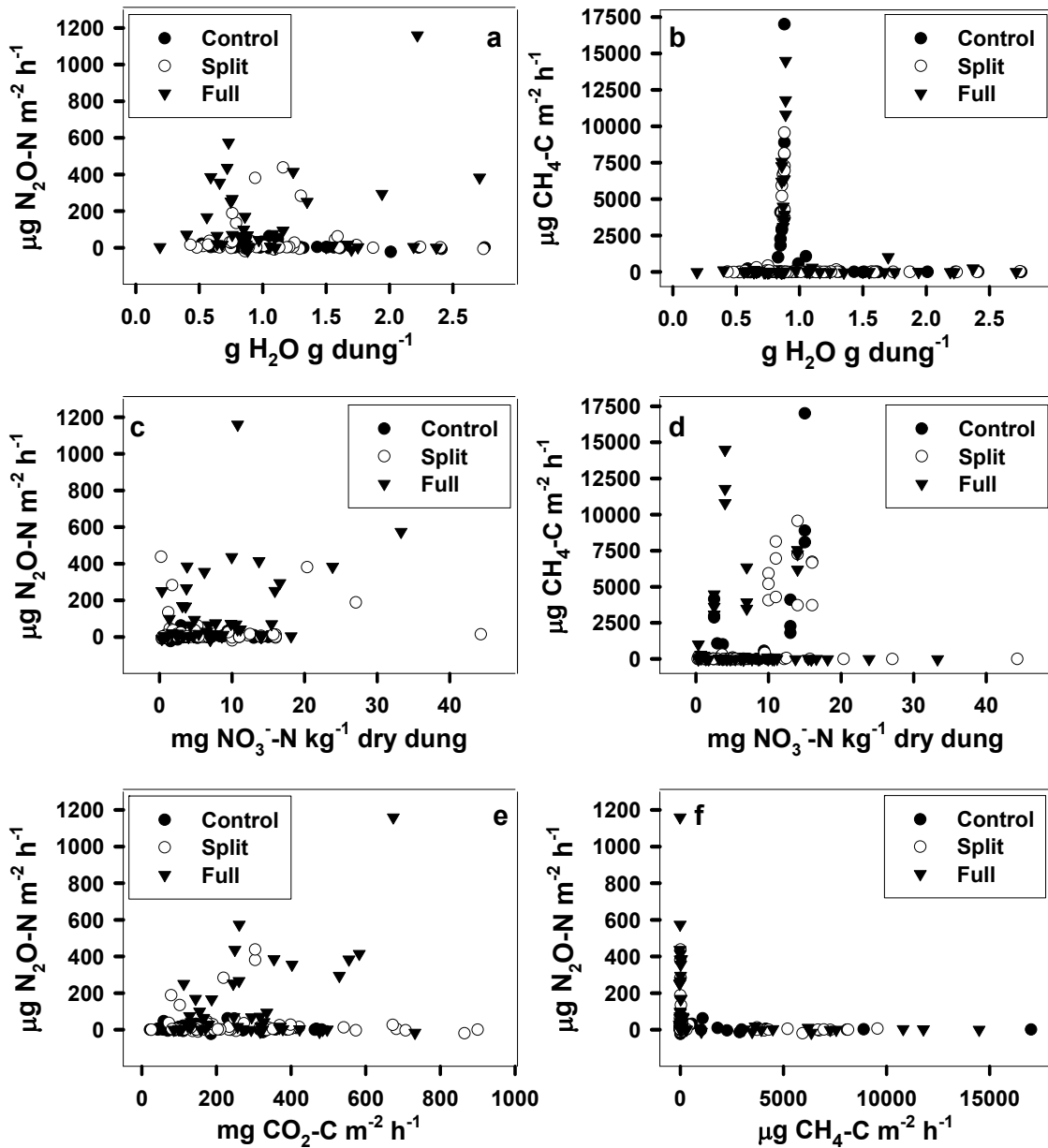


Figure 3.8. Scatterplot graphs illustrating the relationships between independent and dependent variables, as well as between dependent variables. (a) Dung gravimetric moisture content vs. N_2O emission, (b) Dung gravimetric moisture content vs. CH_4 emission, (c) Dung NO_3^- vs. N_2O emission, (d) Dung NO_3^- vs. CH_4 emission, (e) CO_2 vs. N_2O emission, (f) CH_4 vs. N_2O emission.

NO_3^- concentrations, an indication that nitrate was not the most important driver of N_2O emissions (Fig. 3.8, *c*). Methane emissions occurred primarily when dung NO_3^- concentrations were below $18 \text{ mg NO}_3^- \text{-N kg}^{-1}$ (Fig. 3.8, *d*). There was a positive relationship between CO_2 and N_2O gas fluxes, although there were multiple occurrences where very little N_2O was produced during periods of high CO_2 emissions (Fig. 3.8, *e*). There was a very obvious pattern between dung CH_4 and N_2O emissions, with very little N_2O emissions when CH_4 emissions were occurring and vice-versa (Fig. 3.8, *f*).

3.4.2. Urine Experiment

3.4.2.1. Gas Emissions. In all the replicates of this experiment, more N_2O was emitted from artificial urine patches compared to the Control patch which was only treated with water (Fig. 3.9). The Control patches emitted very little N_2O . The driest replicate (Replicate 1) produced the most N_2O emissions and these emissions started a day after application. In this same replicate, N_2O emissions were highest for the Medium and High treatments, although peak emissions from the High treatment were delayed 20 days compared to the other two treatments. The Medium and High treatments typically produced the highest N_2O emissions for Replicates 2 through 5. Emissions were increasingly delayed following application progressing from Replicate 1 to 5. There was a spike in emissions from the High treatment in Replicate 4 on July 26, 2005 (DOY 207). Emissions of N_2O returned to background by the time the experiment ended, August 31, 2005 (DOY 243). By observation, there was an effect of soil moisture in decreasing N_2O emissions. Thus, an Analysis of Covariance was done to elucidate actual urine treatment effects on the cumulative N_2O emissions. The Control treatment produced the least amount of N_2O , and emissions increased from Low, Medium to High urine treatments

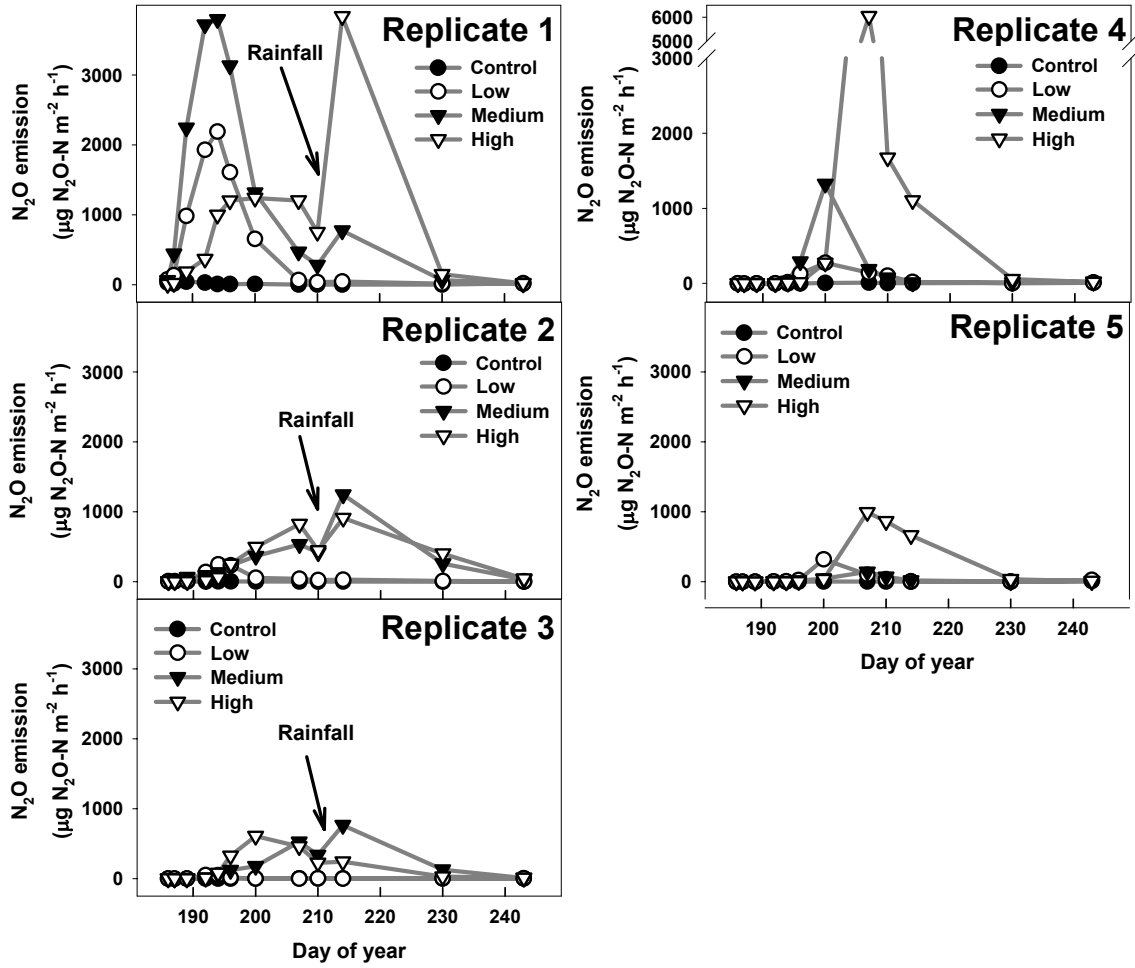


Figure 3.9. Nitrous oxide emissions from all replicates of the Urine Experiment. Values represent data from a single sample position. Note difference in scale for Replicate 4; N₂O data. (Replicate 1 = driest, Replicate 5 = wettest)

(Table 3.6). Control treatment produced fewer emissions than all urine treatments, and the High treatment produced more emissions than the Low treatment.

Differences in CH₄ emissions between the replicates were even more drastic than the differences in N₂O emissions between the replicates (Fig. 3.10). All treatments produced negligible or negative CH₄ emissions in the first and second replicates. The third replicate produced some CH₄ emissions from the High and Low treatments, while very high CH₄ emissions were observed from the High and Medium treatments in Replicate 4. In Replicate 5, all treatments, including the control, produced very high CH₄ emissions. The highest observed fluxes of CH₄ occurred on July 13, 2005 (DOY 194). The high CH₄ emissions observed on Replicates 3, 4 and 5 coincided with a burst of CH₄ emitted from Replicate 1 of the forage plot study (Chapter 2). There were no significant differences between the treatments for cumulative CH₄ emissions (Table 3.6).

3.4.2.2. Other Parameters Measured. This study began at a time when soil conditions were very wet due to an unusual rainfall that lasted two days and 72 mm of rain fell (Fig 3.11). The rainfall occurred June 29 and June 30 (DOY 180-181), causing standing water on Replicates 4 and 5. There was another heavy rainfall that occurred on July 8 (DOY 189), where 20 mm of rain fell. There was relatively little rainfall for the rest of the experiment which allowed for standing water to disappear. Average daily temperatures were above 13°C throughout the experiment. Two warm periods occurred being between July 6 to July 17 (DOY 187-198) and July 30 to August 8 (DOY 211-220). Soil temperature at each chamber followed the same pattern as air temperature (data not shown).

Soil moisture for Replicates 1 through 3 decreased slightly during the course of

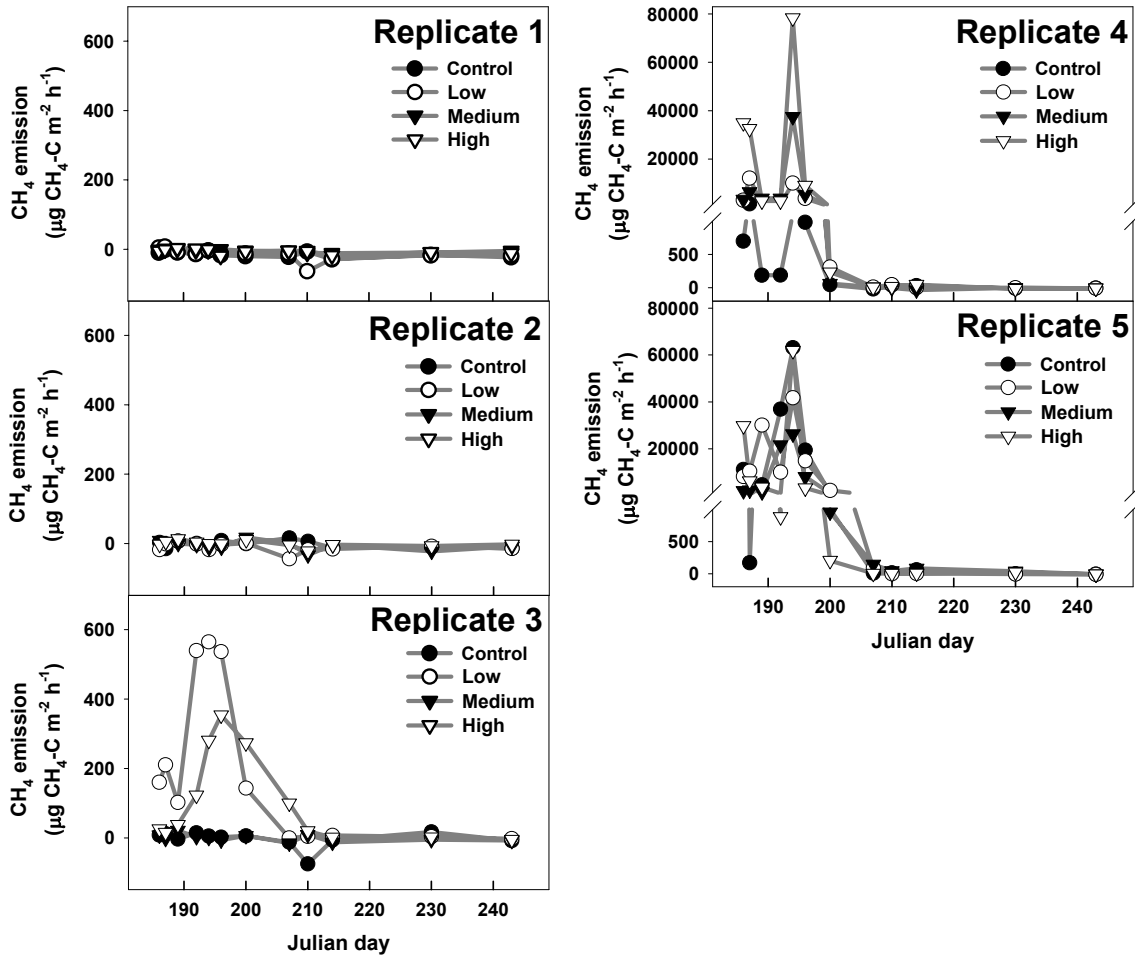


Figure 3.10. Methane emissions from all replicates of the Urine Experiment. Values represent data from a single sample position. Note difference in scale for CH₄ data. (Replicate 1 = driest, Replicate 5 = wettest)

Table 3.6. Cumulative greenhouse gas emissions from artificial urine patches in 2005. Analysis of covariance performed on log transformed data for N₂O and CH₄ emissions. Gravimetric soil moisture content used as covariable. Mean values followed by the same letter are not significantly different (P ≤ 0.05, Tukey). Values for average and standard error represent means of 4 sample positions (only 4 replicates had soil moisture data).

	mg N ₂ O-N m ⁻²		mg CH ₄ -C m ⁻²	
	Average	SE	Average	SE
Control	4 c	2	1800	1800
Low	170 b	110	1600	1600
Medium	550 ab	260	880	890
High	680 a	290	1100	1100

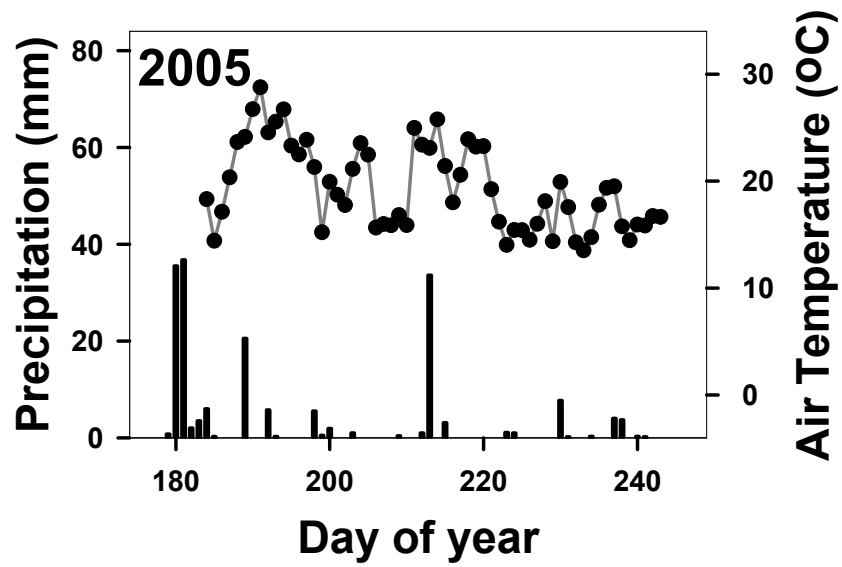


Figure 3.11. Daily total precipitation and average air temperature during the Urine Experiment in 2005. In order to show the occurrence of precipitation prior to the experiments, precipitation data from five days before the start of the experiments is included.

the experiment (Fig. 3.12). For Replicate 5, soil moisture increased on the last sample date, due to a recent rainfall. Soil moisture levels increased from Replicates 1 through to Replicate 5.

The application of artificial cattle urine increased soil NH_4^+ concentrations, although the increase was delayed for wetter replicates (3 and 5) than drier replicates (1 and 2) (Fig. 3.12). Throughout the experiment, Replicates 3 and 5 generally had soil NH_4^+ concentrations that were lower than the other two replicates. There were no apparent differences in soil NH_4^+ concentrations between the urine treatments.

Generally, urine treatment increased soil NO_3^- concentrations, although the increase was progressively smaller from Replicates 1 to 5. With the exception of the Medium treatment of Replicate 1, soil NO_3^- concentrations increased during the course of the experiment for Replicates 1, 2, and 3. NO_3^- concentrations did not increase in Replicate 5 except on the last sample date for the High treatment. Soil NO_3^- concentrations were much lower for Replicates 3 and 5 compared to 1 and 2.

The application of urine increased soil nitrite (NO_2^-) concentrations from undetectable to a max of $92 \text{ mg NO}_2^- \text{-N kg}^{-1}$ in three of the four sampled replicates (Fig 3.13). Replicate 5 did not have detectable NO_2^- in any of the treatments. Nitrite concentrations for all urine treatments decreased from Replicates 1, 2, 3 to 5. In Replicate 1, there was much more soil NO_2^- for the Medium and High urine treatments. For Replicate 2, the High treatment had appreciable NO_2^- concentrations ($>20 \text{ mg N kg}^{-1}$ dry soil) up to day 207. Nitrite was undetectable in the Control treatment.

3.4.2.3. Relationship between Soil Parameters and Gas Emissions. Many of the variables measured in this experiment had very strong correlations to other variable

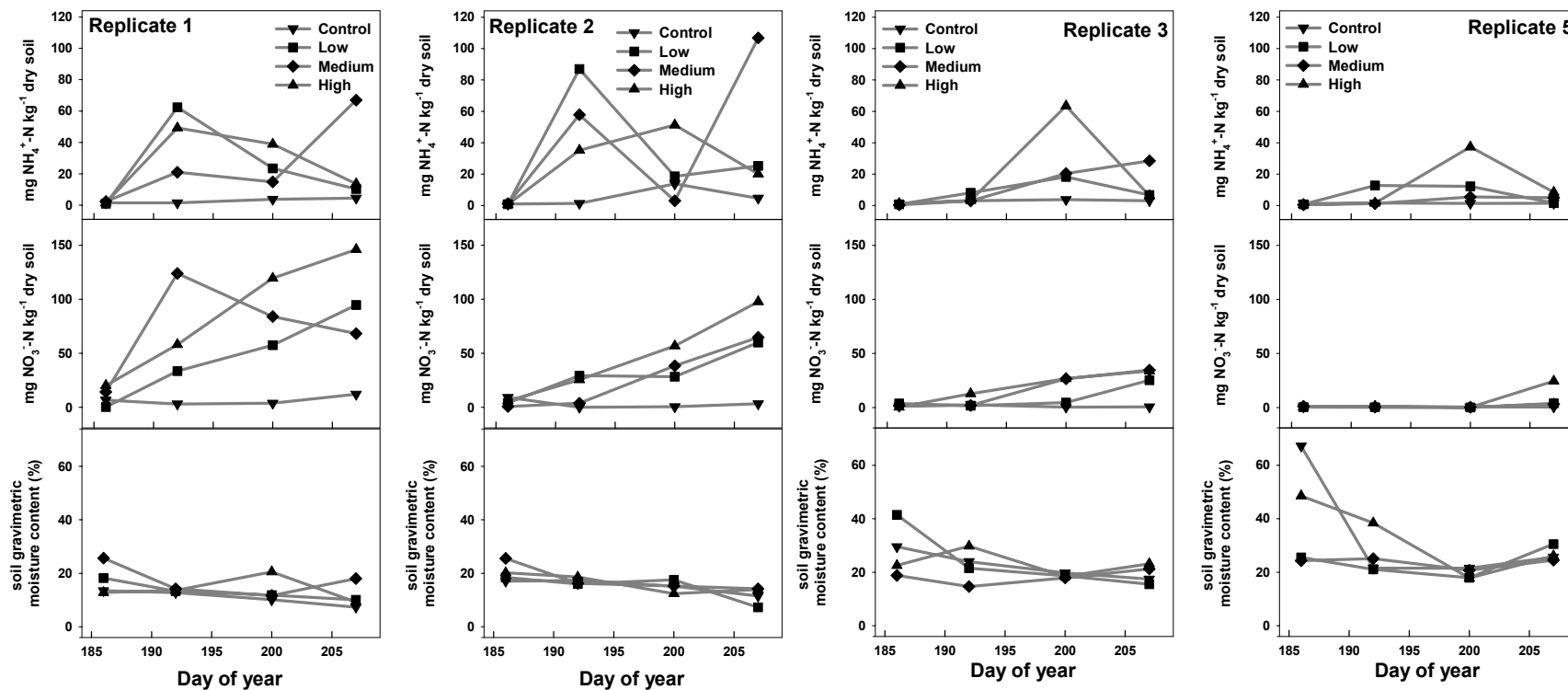


Figure 3.12. Soil ammonium and nitrate concentration, and soil moisture content Replicates 1, 2, 3 and 5 of the Urine Experiment. Values represent data from a single sample position.

Note: Due to a lack of artificial urine, only four out of five replicates were used for soil sampling.

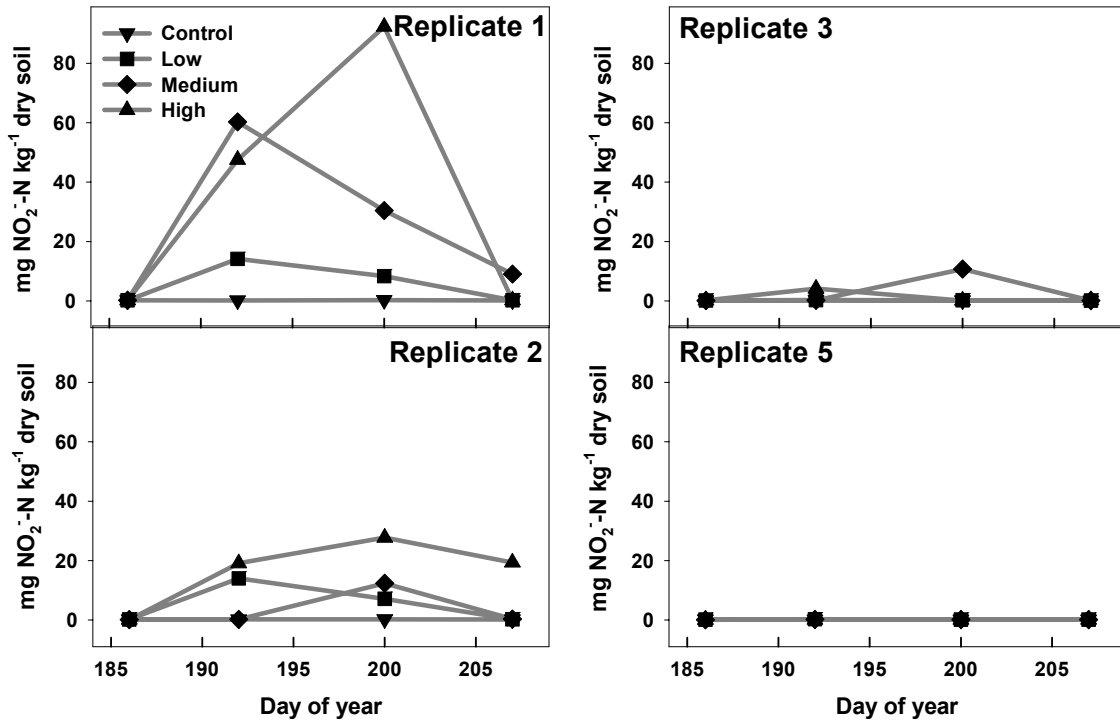


Figure 3.13. Soil nitrite concentrations from Replicates 1, 2, 3 and 5 of the Urine Experiment. Values represent data from a single sample position.
Note: Due to a lack of artificial urine, only four out of five replicates were used for soil sampling.

with relatively high correlation coefficients (r-value) (Table 3.7). The power of these correlations were also very good, with the probability level approaching zero when correlation coefficients were higher than 0.32. Nitrous oxide emissions were positively correlated with soil NH_4^+ , NO_3^- and NO_2^- , and was negatively correlated with CH_4 emission and soil moisture. Methane emissions were positively correlated with soil moisture, and was negatively correlated with soil NH_4^+ , NO_3^- and NO_2^- . In addition, soil NH_4^+ was positively correlated with soil NO_3^- , soil NO_2^- and soil temperature. Soil NH_4^+ and NO_3^- and NO_2^- were negatively correlated with soil moisture.

The relationship between N_2O emission and soil concentrations of NO_3^- and NO_2^- are shown in Figure 3.14. Although some N_2O was produced when there were very low concentrations of soil NO_3^- , the majority of emissions were produced when soil NO_3^- concentrations were above 20 mg N kg^{-1} dry soil. The relationship between soil NO_2^- and N_2O emissions was similar, although there were more N_2O emissions at lower concentrations of soil NO_2^- . In addition, only Medium and High treatments produced soil NO_2^- concentrations above 15 mg N kg^{-1} dry soil.

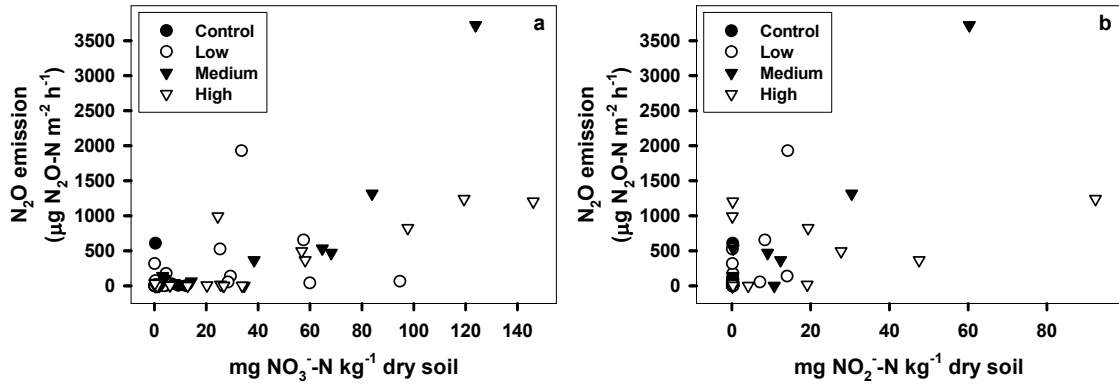
3.5. Discussion

There are no studies that have examined the effect of manure fertilized grassland pasture on the greenhouse gas emissions of cattle dung and urine patches excreted by cattle grazing these forages. In addition, there are few studies examining the effect of soil moisture conditions on the quantity and type of greenhouse gas emission from cattle dung and urine patches. The results from this study will help to fill a knowledge gap regarding greenhouse gas emissions from fertilized grassland pasture systems.

In a separate study on the same research site, forage samples were collected from

Table 3.7. Spearman Correlation Coefficients and probability level for the Urine Experiment (both replicates and both years; n=61). Values in bold show strong correlation between two variables.

	N₂O	CH₄	NH₄⁺	NO₃⁻	NO₂⁻	gmc¹	soil temp.
N₂O	1.00000						
CH₄	-0.41502 0.0009	1.00000					
NH₄⁺	0.58339 ≤0.0001	-0.32822 0.0098	1.00000				
NO₃⁻	0.56535 ≤0.0001	-0.64526 ≤0.0001	0.58678 ≤0.0001	1.00000			
NO₂⁻	0.46212 0.0002	-0.36731 0.0036	0.51741 ≤0.0001	0.58211 ≤0.0001	1.00000		
gmc¹	-0.49116 ≤0.0001	0.60797 ≤0.0001	-0.44284 0.0004	-0.4791 ≤0.0001	-0.38379 0.0023	1.00000	
soil temp.	0.04544 0.7280	0.15927 0.2202	0.27346 0.0330	-0.06462 0.6208	0.00716 0.9563	0.07831 0.5486	1.00000



the grazed plots in order to determine forage yield and nutrient content (Wilson 2007). The forage in the Split and Spring treatment pastures had 1.77 and 2.04 times more N, respectively, than the Control pastures. As grass generally contains an excess of N for grazing animals, the increase in N concentration of grass due to fertilization should cause an increase in urinary and fecal excretion of N from cattle. This increased excretion of N in turn could have an impact on greenhouse gas emissions from dung and urine patches.

The results of the studies presented in Chapter 2 show a clear impact of soil moisture on the quantity and type of greenhouse gases produced from grassland after manure application. Drier areas of the landscape produced more N₂O, while only saturated areas produced CH₄. This same pattern may exist for dung and urine patches, although very little research has been done to show this.

3.5.1. Effect of Manure Application on N₂O Emissions from Dung and Urine Patches

3.5.1.1. Dung. The cattle dung produced N₂O emissions that were comparable to emissions from their respective grassland plots, although emissions from dung patches were often higher (max. average of 78 µg N₂O-N m⁻² h⁻¹ in Split grassland plots vs. 243 µg N₂O-N m⁻² h⁻¹ from Split dung patches). In addition, the cumulative emissions of N₂O from the dung patches were high for a short period of sampling. Emissions of N₂O should have continued after the sampling periods as microbial processes would have continued in the patch for some time after sampling had ceased. Studies by Allen et al. (1996), Flessa et al. (1996) and Yamulki et al. (1998) all show that N₂O emissions from cattle dung patches can be produced over a period of several months. One study by Maljanen et al. (2007) only shows a rapid increase of N₂O emissions four weeks after

dung application. These field studies all show that N_2O emissions from cattle dung patches are higher than from a control grassland soil even though the control grassland examined by Maljanen et al. (2007) was fertilized with commercial N fertilizer.

A novel aspect of this study is the effects of manure fertilization to grass pasture on N_2O emission from cattle dung patches. In addition, to my knowledge no studies have made observations concerning a relationship between the nutrient content of cattle dung and greenhouse gas emissions. Manure application to the pastures had the effect of producing cattle dung with larger N_2O emissions. As was hypothesized, dung from the Spring treatment produced the highest amount of N_2O emissions in both years of the study. The forage sample results show that the N content of the forage on the Spring treatment was twice as high as the forage N content from the Control pastures. In addition, the dung samples collected from the Spring pastures were generally higher in total N than the dung collected from the Control and Split pastures. Dung from the Split treatment produced considerable amounts of N_2O in 2004, but very little in 2005. In addition, there was a decrease in dung N_2O emissions from the first replicate of the Spring treatment. Environmental conditions may have limited the N_2O emissions from many dung patches in 2005, as high N concentrations in forage and dung samples contradict the reduction in N_2O emissions. Dung patches may have remained wetter throughout 2005, even though the soil was not saturated as in 2004. This may have caused a more prolonged emission of CH_4 from the dung patches in 2005, and may have had an impact on N_2O emissions as well. Higher dung moisture contents in 2005 may have increased the N_2/N_2O ratio being produced from the patches, and only the driest dung patches (Spring treatment, Replicate 2) produced large amounts of N_2O emissions.

3.5.1.2. Urine. As hypothesized, the addition of artificial cattle urine caused a very large increase in N₂O emissions from the grassland soil. Several studies have shown that urine from grazing animals can stimulate N₂O emissions from soil (Allen et al. 1996, Flessa et al. 1996, Sherlock and Goh 1983, Yamulki et al. 1998). In this study, no immediate emission of N₂O was detected after the urine deposition, as was seen in the study by Sherlock and Goh (1983). The reason that N₂O emissions are delayed from urine patches is that time is required for urea to hydrolyze to NH₄⁺ for nitrification and denitrification to occur.

In terms of composition, the makeup of the artificial cattle urine used in this experiment was representative of real cattle urine. The range of N concentration used in this experiment was within ranges reported by De Boer et al. (2002), Bristow et al. (1992) and Oenema et al. (1997), although the concentrations used were too high due to a calculation error. The N concentrations used may have been more suited to dairy cattle than grass-grazed beef cattle. Of particular importance was the addition of hippuric acid to the artificial urine solutions. Hippuric acid (HA) in cattle urine has been proven to be a natural inhibitor of soil N₂O emissions (Kool et al. 2006a, Kool et al. 2006b and Van Groenigen et al. 2006). We did not change the concentration of 4 g per liter of HA (0.31 g/L HA-N) across all the treatments. This is important because increasing concentrations of HA in cattle urine has the effect of decreasing denitrification rates and thus N₂O emissions (Kool et al. 2006a and Van Groenigen et al. 2006). The HA concentration used in this study was at the low end of a range studied by Kool et al. (2006a), and the real HA concentration of the urine from the cattle present on the study site may have been higher.

There are several reports in the literature that cite that either nitrification (Monaghan and Barraclough 1993) or denitrification (Sherlock and Goh 1983, Van Groenigen et al. 2005a) as the major source of N₂O emissions from urine patches. Some other studies report that N₂O emissions occur during both nitrification and denitrification processes (e.g. Allen et al. 1996). There is evidence that both nitrification and denitrification are responsible for the N₂O emissions from the urine patches in this study. First, there was a strong correlation between N₂O emission and both soil NH₄⁺ and NO₃⁻ concentrations. Correlation between both soil NH₄⁺ and NO₃⁻ concentrations with N₂O emission indicates that both nitrification and denitrification could be responsible for N₂O emissions (Firestone and Davidson 1989). Secondly, N₂O emissions began the day after urine deposition in Replicate 1, indicating that nitrification of NH₄⁺ to NO₃⁻ was a possible source of N₂O emission as this is soon for denitrification derived N₂O emissions to occur (Van Groenigen et al. 2005a). The high concentrations of NO₂⁻ found in the soil after urine deposition also indicate that N₂O fluxes may be originating from nitrification (Oenema et al. 1997), as NO₂⁻ is chemically unstable and readily reacts and is lost as N₂O. Finally, there is evidence of denitrification driving some N₂O emissions, as there was an increase of N₂O emissions in Replicates 1, 2 and 3 after a rainfall event in the middle of the experiment. However, due to limitations of sampling technique it is impossible to positively conclude whether nitrification or denitrification is responsible for N₂O emissions at a particular time and place.

In recent years, there have been more studies examining the effect of urine N concentration and urine composition on N₂O emissions (Van Groenigen et al. 2005a, Van Groenigen et al. 2005b). However, our study is particularly important because it

investigates the potential effect of manure application to grassland pasture on N₂O emissions from cattle urine patches. An effect of urine N concentration on N₂O emission was evident from this experiment, as the Medium and High treatments consistently produced more N₂O emissions over a longer period of time. In a field study by Van Groenigen et al. (2005b), the effects of urine concentration were not as consistent as this study, and effects were only seen on two different application dates. An incubation study by Van Groenigen et al. (2005a) showed no significant effect of four urine N concentrations on N₂O emissions. Higher concentrations of N compounds in the urine should, intuitively, increase N₂O emissions by increasing the concentrations of N compounds in the soil. Higher NH₄⁺ concentrations in the soil should drive higher rates of nitrification, which will supply NO₃⁻ so that higher rates of denitrification can occur when the appropriate conditions exist. In addition, N₂O emissions should occur for shorter periods of time when lower concentration of N are added to soil, as the substrates driving nitrification and denitrification will decrease in concentration faster (Firestone and Davidson 1989).

One interesting occurrence to note from this study is the lack of N₂O emission from the High treatment at the start of experiment on Replicate 1. Lower N₂O emissions from artificial urine patches with higher N concentrations were also observed by Van Groenigen et al. (2005a). There is evidence that nitrification may have been inhibited in this study, as high concentrations of NO₂⁻ indicate that sensitive nitrite oxidizers were slow at producing NO₃⁻. Monaghan and Barraclough (1992) concluded that inhibition of nitrification in near-neutral pH, temperate grassland soils, should only occur when urine-N concentrations exceeded 16 g N/L. It is important to note that although the urine

concentrations in this study were all lower than 16 g N/L, application rates were much higher in this study (~4x per unit area). High concentrations of free ammonia, salts, and high soil pH all contribute to inhibit nitrite oxidizers, and these concentrations can be affected significantly by soil moisture conditions. Thus, the dry, sandy, and high pH soil at this site treated with the High urine treatment could produce conditions with high free ammonia, high osmotic pressure, and even higher pH, thus inhibiting nitrification and reducing N₂O emissions (Monaghan and Barraclough 1992).

3.5.2. Soil Moisture Content Determines Variation of Nitrous Oxide Emissions from Cattle Dung and Urine Patches

Studies of N₂O emissions from cattle dung patches do not show a conclusive relationship between dung moisture and N₂O emissions. A study by Yamulki et al. (1998) produced no correlations between soil moisture and N₂O emissions, although there were some positive correlations with rainfall. Maljanen et al. (2007) have shown a positive correlation between soil moisture and N₂O emissions, with large N₂O emissions at very high water filled pore space (80-90%). Allen et al. (1996) observed that dung patches on a poorly drained clay soil (moisture content 30-50 %) produced much less N₂O emissions than dung patches on a moderately drained loam (moisture content 18-25 %). The range of soil moisture used by Allen et al. (1996) is comparable to the variation in soil moisture content across the plots in this experiment, where most of the N₂O emissions from the cattle dung occurred in the drier locations. This was especially true in 2004, when rainfall brought the water table to the surface, saturating several dung patches. This saturation could provide an environment where N₂O emissions would be inhibited, as the low soil O₂ availability would cause nitrification to be inhibited, and any

denitrification occurring would produce a high N_2/N_2O emission ratio (Allen et al. 1996). The drier conditions in the more elevated dung patches were associated with higher concentrations of NO_3^- (Replicate 2, Full spring treatment). In 2005, conditions were drier at the beginning of the experiment, which would have allowed nitrification rates to be higher for the dung patches located in the depressions of the plots. Increases in N_2O emissions after two rainfalls were most likely due to increases in denitrification rates, when the moisture contents of the dung patches would have significantly increased. Stronger correlations found between N_2O emission and dung NO_3^- concentration (Table 3.5) also indicate that denitrification was a more important process in producing N_2O from the dung patches.

Several studies have examined the effect of soil moisture on N_2O emissions from urine patches, and these studies have also not been conclusive. None of these studies have examined the effect of a moisture gradient on N_2O emissions from urine patches. Maljanen et al. (2007) found that N_2O emissions from urine patches in a field experiment increased with increasing soil moisture. Clough et al. (2004) found in an incubation experiment with a silt loam soil that N_2O emissions from urine patches were higher when soil was saturated than when soil was at field capacity. In this study, higher soil moisture conditions had an impact of lowering emissions of N_2O from the artificial urine patches. These results correspond with an incubation study using urine treated sandy soil by Van Groenigen et al. (2005a), where N_2O emissions were delayed from saturated soil until soil moisture was reduced. Saturated soil conditions inhibit nitrification and thus denitrification of the product of nitrification, NO_3^- (Malhi and McGill 1982). This delay could allow grasses to uptake the excess soil NH_4^+ after urine deposition. Saturated

sandy soils also allow diffusion of nitrogen from the urine patch to the surrounding soil, reducing N concentrations in the patch and lowering N₂O emissions per unit area. The slower increases in concentration for soil NO₃⁻ is evidence that high soil moisture content is inhibiting the nitrification process in the wetter soils, or there is a high degree of loss via denitrification. Drier soil conditions, which produced larger N₂O emissions in this experiment, were associated with higher concentrations of soil NH₄⁺, NO₃⁻ and NO₂⁻, and a faster rate of increase of NO₃⁻.

3.5.3. Methane and Carbon Dioxide Emissions from Dung and Urine Patches

Large emissions of CH₄ were produced from all the dung patches regardless of treatment. These emissions were much higher than the surrounding grassland soil surface during the sampling periods. These results were expected as several studies have found that cattle dung patches can be important sources of CH₄ (Flessa et al. 1996, Holter 1997, Jarvis et al. 1995, Yamulki et al. 1999).

Methane emissions from the cattle dung patches occurred mostly within 10 days of deposition. Studies have shown that this is typical for cattle dung patches (Flessa et al. 1996, Holter 1997, Jarvis et al. 1995, Yamulki et al. 1999). There was a large difference in the peak and duration of CH₄ emissions from the dung patches in 2004 and 2005. The warm, dry, sunny conditions during the deposition of the dung in 2004 may have contributed to the smaller emissions occurring over a shorter period of time. Holter (1997) observed that dry conditions cause cattle dung patches to dry out more quickly, creating aerobic conditions in the patch, halting CH₄ emissions. Yamulki et al. (1999) hypothesized that the formation of a crust during drying may decrease CH₄ volatilization, which would increase the potential for CH₄ oxidation. A heavy insect disturbance (dung

beetles and flies) observed in 2004 may also have increased the drying throughout the patch, effectively aerating the dung patches. The experiment in 2004 was started after the cattle had already been removed from the site, thus there was no fresh dung for these particular insects to feed on. The 2005 dung experiment was started when cattle were still on the research site, and we did not see the level of insect activity seen in 2004. The cooler conditions at the beginning of the experiment in 2005, in addition to the lack of insect disturbance, most likely contributed to higher CH₄ emissions from the dung patches. Yamulki et al. (1999) observed increased cumulative CH₄ emissions when experiments were conducted with higher rainfall and lower air temperatures.

The spatial variability of CH₄ emissions from the dung patches was not as great as the N₂O emissions. This was especially true during the beginning of the experiments. In contrast to the N₂O emissions, all the dung patches produced CH₄ emissions at the beginning of the experiments. Soil moisture conditions did not seem to have an effect on the emission of CH₄ during this period. However, once the initial burst of CH₄ emissions had decreased, there were some patches that continued to produce CH₄ emissions that were much smaller than following deposition. These smaller emissions were only observed in areas of saturated soil conditions, where the water table was at or near the soil surface. These small emissions contradict a study by Holter (1997), who reported that there was no CH₄ emissions after rewetting of dung patches.

There have been no studies examining the effect of manure-fertilized grassland pasture on CH₄ emissions from cattle dung. An increase in CH₄ emissions was expected from the dung patches originating from the manure-treated pastures, compared to the dung from the Control pastures. Jarvis et al. (1995) have reported that cattle dung with a

lower C/N ratio produced higher amounts of CH₄ emissions, and the dung produced in the manure-treated pastures definitely had higher N concentrations and, presumably, lower C/N ratios (no C analysis was done). There were larger emissions in 2004 from the Split and Spring treatment dung patches compared to the Control treatment, although in 2005, there were no definite differences between the treatments. The lack of significant differences between treatments may be due to the lack of replication in this study, and also to the very high emissions observed in 2005, which masked the differences observed in 2004.

Few studies have examined carbon dioxide emissions from cattle dung patches. In this study, CO₂ emissions from the cattle dung were generally higher than from the grassland soil surface. Cattle dung patches are hot spots of available C and other nutrients, and are able to hold moisture to support microbial growth. Increased microbial respiration in these patches caused large amounts of CO₂ to be emitted from the patch. In both years, high emissions of CO₂ were observed soon after dung deposition. Generally, there were higher CO₂ emissions from dung patches produced from cattle pastures treated with liquid hog manure. The increase in CO₂ emission could be caused by an increase in available C in these patches, as well as a lower C/N ratio, which would both increase the microbial respiration from the patch. For example, a study by Eiland et al. (2001) found that straw mixed with manure lowered the initial C/N ratio and produced higher CO₂ emissions.

Studies by Flessa et al. (1996), Lovell and Jarvis (1996) and Yamulki et al. (1999), have shown that cattle urine patches are not important sources of CH₄, and that they can act as sinks of CH₄. Considerable CH₄ emissions were observed in the lower, wetter

replicates of this experiment. Although these emissions did not occur from the control in Replicates 3 and 4, they did occur from the control in Replicate 5. These CH₄ emissions do not appear to be associated with the addition of the artificial cattle urine. Any effect of urine deposition on CH₄ emission from a sandy soil would be expected to be short-lived, as the soil drains quickly, and available C concentrations in urine are not very significant (compared to dung for example). Secondly, these very high CH₄ emissions occurred at the same time as very high CH₄ emissions from the plot experiment of this study. Thus, the CH₄ emissions produced were caused by conditions existing in the soil without urine application (saturated soil, C source from roots and soil).

3.6. Summary

This study has shown that the deposition of cattle dung is an important source of N₂O, CH₄ and CO₂. Although important, N₂O emissions from dung patches were not as great as urine patches. In addition, fertilization of cattle pasture with liquid hog manure had an effect on cumulative N₂O and CO₂ emissions from cattle dung, but did not have an effect on cattle dung CH₄ emissions. Finally, there was also a significant increase in radiative gas flux when dung N₂O and CH₄ emissions were combined as CO₂ equivalents.

Nitrous oxide emissions from urine patches are very important sources of N₂O in cattle pastures. Cattle dung is also an important source of N₂O, although not nearly as important as urine patches. For example, cumulative urine N₂O emissions were an average of 170 ± 110 and 680 ± 290 mg N₂O-N m⁻² for the Low and High urine treatments, respectively. In contrast, cumulative dung N₂O emissions in 2005 were much lower: an average of 4 ± 2 and 87 ± 33 mg N₂O-N m⁻² for the Control and Spring treatment dung, respectively. As urine is a more important source of N₂O emission on

cattle pasture, the impact of increasing N_2O emissions from dung will be less. In contrast, emissions of CH_4 were not affected by urine treatment at all, and forage treatment did not affect the emission of CH_4 from the dung patches.

The cattle grazing manure-fertilized forage in this study had higher blood urea N levels, which would be reflected by the urea levels in the urine. Urine patches in particular have been shown in this study to be very large sources of N_2O , and are very important when considering the total N_2O emissions from cattle pastures. There were no significant effects of artificial cattle urine deposition to soil CH_4 and CO_2 emissions. The deposition of artificial cattle urine with higher concentrations of N significantly increased the emissions of N_2O . Thus, cattle that were grazing the manure-treated pastures were most likely excreting urine that produced higher N_2O emissions than the cattle consuming non-fertilized forage. This increase would be important when considering total N_2O emissions from cattle pastures, as urine patches are large sources of N_2O .

The amount of N_2O emissions from cattle dung and urine patches is greatly dependent on soil moisture conditions and thus, landscape position. Nitrous oxide emissions were most important in the more elevated, drier areas of the landscape. However, there was a delay in N_2O emissions for the High urine treatment on the driest replicate. This may be due to a toxic effect of salts, high pH and high ammonia concentration caused by the addition of the High urine treatment. In addition, high soil NO_2^- concentrations on the drier replicates indicate that nitrification was an important source of N_2O from the artificial urine patches. The wetter, lower positions of the landscape produced much less N_2O . Methane emissions from dung patches are important; however these emissions were less dependent on soil moisture. All the dung

patches produced methane soon after deposition, regardless of landscape position. The findings regarding N₂O emissions from dung and urine patches at different landscape positions are important when considering the management of grassland pasture systems.

This study has highlighted some important knowledge gaps in terms of the spatial variability of GHG emissions from cattle dung and urine patches. It has also demonstrated a feedback mechanism that exists in a cattle pasture system when nutrients are added in the form of manures. This feedback mechanism is particularly important when considering N₂O emissions from dung and urine patches, as these are very important sources of N₂O in cattle pastures.

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4. DISCUSSION

4.1 Salient Points

This study was novel in many ways, and as such it contributed a great deal to Canadian and global research concerning greenhouse gas emissions from agriculture. One unique aspect of this study was that season-long measurements were taken from perennial grassland plots. Soil greenhouse gas studies in Canada have typically focused on cultivated soils and annual crops, and these studies are typically focused on a particular portion of the growing season. This study also examined the effect of manure application timing, with a novel aspect being the study of a split application on greenhouse gas emissions. Finally, effects of soil moisture variability on greenhouse gas emissions were examined, and this portion of the study produced some of the most important results of the entire study.

Liquid hog manure application to grassland plots had the effect of increasing both N₂O and CH₄ emissions. Although a significant increase was only observed with N₂O, this increase was large enough to cause a significant increase in CO₂ equivalents when the greenhouse gas potentials of N₂O and CH₄ were combined. A particularly important finding is that cumulative N₂O emissions from the Spring treatment plots were significantly higher than the Split treatment plots. This difference is assumed to be due to lower soil temperatures after fall manure application, and to a lower potential for high NO₃⁻ concentrations. Spring thaw emissions, which would contribute to N₂O emissions from the Split treatment, were not recorded. However there was no evidence that these

emissions occurred, as there was little soil NO_3^- from the Split treatment plots in the spring.

Cattle dung and urine were shown to be important sources of greenhouse gases in this study. Cattle dung was a source of N_2O , CH_4 and CO_2 emissions, while the artificial cattle urine stimulated the production of high levels of N_2O . Manure application to the pastures had the effect of significantly increasing N_2O and CO_2 emissions from cattle dung. The artificial cattle urine was shown to be a much more important source of N_2O emission than cattle dung. Increasing artificial urine N concentration significantly increased N_2O emissions from the urine patches. This research has shown evidence of a feedback mechanism where liquid hog manure application to pastures can cause an increase in greenhouse gases from cattle dung and urine patches.

The characteristics of the site in combination with the very wet conditions of 2004 and 2005 were ideal because it allowed us to observe greenhouse gas emissions from areas with contrasting soil moisture contents. Soil moisture was shown to be a major factor controlling the type and quantity of greenhouse gas emissions originating from the grassland soil surface, and from cattle dung and urine patches. In general, saturated conditions produced CH_4 from the grassland soil surface, while drier soil conditions were net sinks of CH_4 . Drier conditions caused an increase in N_2O emissions, especially after a rainfall or soon after manure application. The same effect was shown with urine patches, although CH_4 emissions were not increased by urine application. For the cattle dung, drier conditions caused an increase in N_2O emissions; however, saturated conditions did not have an important impact on CH_4 emissions, as all dung patches initially produced some CH_4 . The finding that grassland under saturated conditions can

produce CH₄ emissions is important, as generally, agricultural soils are considered to be sinks of CH₄. However, grasslands used for pasture are often marginal due to many factors, one of them being wet soil conditions. Grasslands and pastures that are susceptible to saturated soil conditions should now be considered as potential sources of CH₄ in greenhouse gas budgets. This represents a significant amount of agricultural land in Canada, as much of the land on the northern and eastern fringe of the prairies is limited to pasture and hay production due to excessive soil moisture conditions (e.g. South-Eastern Manitoba, Interlake).

4.2 The Big Picture: Soil Surface, Dung and Urine Greenhouse Gas Emissions

If a system is large and consists of many different parts, all parts of that system should be examined before any management practice is recommended. Thus, in order to determine the total C budget from a cattle pasture, greenhouse gas emissions from the soil surface, from cattle dung and urine patches and from cattle themselves (CH₄ from enteric fermentation) must be examined along with the potential for C sequestration in the landscape itself.

Although cattle dung and urine patches cover relatively small areas of a total pasture area (1% and 2.4%, respectively (Flessa et al. 1996)), they have been proven in this study and others to be important sources of atmospheric N₂O. Urine patches in particular have been shown to produce particularly high N₂O emissions. The average cumulative N₂O emission from the Low urine patches (corresponding to cattle grazing the Control pastures) in this study was 169 mg N₂O-N m⁻². Assuming that these urine patches cover 2.4% of the pasture, if spread evenly throughout a pasture would amount to 4.06 mg N₂O-N m⁻². This is almost 39 % of the cumulative N₂O measured from the

Control hay plots. In comparison, the average cumulative N₂O emission from the High urine patches (corresponding to cattle grazing the Spring treatment pastures) is equivalent to almost 9% of the cumulative N₂O measured from the Spring treatment hay plots. These numbers are high considering that emissions were only measured from the urine patches over a period of about two months. In contrast, assuming that dung patches cover 1% of pasture, cumulative N₂O emissions from the dung patches in this study is equivalent to less than 1% of the cumulative N₂O emissions from the hay plots. These numbers are similar to what was found by Flessa et al. (1996), who reported that dung patches were much less important than urine patches when considering N₂O emission from pasture. As for CH₄ emissions from cattle dung, cumulative emissions were equal to between 0.7 and 1.7% of the cumulative CH₄ emissions from the hay plots in Ch. 2. CH₄ emissions from cattle dung should be more important than these numbers indicate, as the CH₄ emissions from the hay plots were particularly high during the years of this study.

Considering the relative importance of urine-derived N₂O from pastures, the significant increase in N₂O emissions produced from the higher concentrations of urine will have a large effect on total N₂O emissions from pasture. In addition, the significant increase in N₂O emissions from the cattle dung originating from manure-treated pastures should also have an effect. In pasture systems, CH₄ emissions from cattle dung are relatively unimportant compared to enteric emissions from adult cattle. Flessa et al. (1996) found that cattle dung represented about 0.778 g CH₄-C per animal per day, while cattle at the La Broquerie site produced 134, 110 and 122 g CH₄-C per animal per day from the Control, Split and Spring pasture treatments, respectively (Wilson 2007). Thus,

the impact of an increase in CH₄ from cattle dung due to manure application would be minimal compared to enteric emissions.

In order for a greenhouse gas budget to be complete for this study, a thorough analysis of carbon sequestration must be done. As was shown in Fig 2.17, a substantial increase in above- and below-ground biomass occurred after the second year of manure application to the Spring treatment plot. Of particular importance is the increase in the below-ground biomass, as this could be an indication of carbon sequestration in manure-treated grassland. The application of manure to the Spring treatment plot over two years caused an increase in root mass of 45% in the top 5 cm of soil compared to the Control plot. Assuming that the soil surface has a density of 1.2 g kg⁻¹, this represents an increase of 10.2 tonnes of dried root mass per ha, a substantial amount of carbon. A review of greenhouse gas budgets from nine European grassland sites (grazed and cut; organic fertilizer, inorganic fertilizer and no fertilizer) demonstrates that the majority of sites produced a negative net greenhouse gas balance (Soussana et al. 2007). The reason these sites have a negative greenhouse gas balance is that the carbon that is stored every year is more important than the greenhouse gases produced by the system. Although greenhouse gases emitted from a grassland pasture as a result of manure application are important, increases in soil carbon may outweigh the effect of those greenhouse gases on the net greenhouse gas budget. The importance of this stored soil carbon is probably even more important at a site such as this, as the site is particularly nutrient poor and unproductive without fertilization.

A master's student is currently studying the data from this thesis, as well as the greenhouse gas data from cattle sampled on the same site over the same time period. The

purpose of this work is to produce a complete greenhouse gas budget for cattle on pasture as affected by the different treatments. This work will better determine the importance of emissions from cattle, the soil, and dung and urine patches in the cattle pasture system.

4.3 Future Work

The soil moisture and greenhouse gas data from this study can be included in a model to predict the emission of N_2O and CH_4 from grassland. However, it is important to note that this study was conducted on an extremely coarse-textured soil with a highly fluctuating water table. Soil texture would likely be required in a model predicting greenhouse gases, as this property has a large influence on soil moisture and soil nutrients. Finally, a topographic survey of the research site conducted by Prairie Farm Rehabilitation Administration (PFRA) should be combined with the greenhouse gas results from this study in order to model greenhouse gas emissions from the entire site.

Additionally, archived soil samples from this study should be analyzed for dissolved organic carbon to observe how this variable impacted soil greenhouse gas emission. This readily-available source of carbon is found in liquid hog manure and can increase rates of both denitrification and methanogenesis. The impact of liquid hog manure on soil dissolved organic carbon later in the season should also be investigated. An increased root mass due to manure fertilization should produce more root exudates and more microbial activity, thus increasing soil dissolved organic carbon.

While there was sufficient data to show a treatment effect of manure application on dung and urine greenhouse gas emissions, more research is required to acquire data that is robust enough to use in modeling. In order to produce data for modelling, a detailed transect study should be conducted in order to observe more closely the impacts

of soil moisture content on the GHG emissions from cattle dung and urine patches. In addition, a more robust study with more replication should be conducted on both types of patches and a diurnal study of GHG emissions from these patches should be conducted in order to observe how emissions change over day and night. The former study should be conducted over a much longer period of time, and started at different times of the year, in order to obtain the most complete picture of greenhouse gas emissions from excreta patches as possible.

Finally, as manure application was shown to increase the root density in the top 5 cm of soil, detailed soil samples (down to 120 cm) should be taken to obtain results for root density and total carbon. The lability of the stored carbon needs to be examined as well, as much of this carbon is stored near the soil surface, and may quickly be respired by soil organisms. This data is crucial, as any increase in greenhouse gases originating from the soil surface, cattle enteric emissions or from cattle excreta may be nullified by the C that is stored in the soil after manure application to grassland.

4.4 References

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