University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Theses, Dissertations, and Student Research in Agronomy and Horticulture

Agronomy and Horticulture Department

12-2012

Nitrous Oxide Emissions from Smooth Bromegrass Pasture under Nitrogen Fertilizer and Ruminant Urine Application in Eastern Nebraska

Laura K. Snell
University of Nebraska-Lincoln

Follow this and additional works at: http://digitalcommons.unl.edu/agronhortdiss

 Part of the Agricultural Science Commons, Agriculture Commons, and the Agronomy and Crop Sciences Commons

Snell, Laura K., "Nitrous Oxide Emissions from Smooth Bromegrass Pasture under Nitrogen Fertilizer and Ruminant Urine Application in Eastern Nebraska" (2012). *Theses, Dissertations, and Student Research in Agronomy and Horticulture*. 60.
http://digitalcommons.unl.edu/agronhortdiss/60

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Theses, Dissertations, and Student Research in Agronomy and Horticulture by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Nitrous Oxide Emissions from Smooth Bromegrass Pasture under Nitrogen Fertilizer and Ruminant Urine Application in Eastern Nebraska

By

Laura K. Snell

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Professor John A. Guretzky

Lincoln, Nebraska

December, 2012

Nitrous Oxide Emissions from Smooth Bromegrass Pasture under Nitrogen Fertilizer and Ruminant Urine Application in Eastern Nebraska

Laura K. Snell, M.S.

University of Nebraska, 2012

Advisor: John A. Guretzky

Nitrous oxide (N_2O) is a greenhouse gas primarily produced in soils by denitrifying and nitrifying organisms. In terms of global warming potential (GWP), N_2O has 310 times the GWP of carbon dioxide (CO_2). Agricultural soils account for 70% of emissions in the United States, but little data is available for contributions from managed pasture ecosystems. This study focused on the production of N_2O in smooth bromegrass (*Bromus inermis* Leyss.) pastures established on silt loam soils in eastern Nebraska. Thirty smooth bromegrass plots (1.5m x 1.5m) were treated with five different fertilizer treatments (0, 45, 90, 135, and 180 kg N/ha) and two urine treatments (urine and no urine). Herbage sampling was taken the day before sampling by clipping the grass within the anchor to a 10 cm stubble height and oven drying the samples. In 2011, a significant effect between the urine treatment x fertilizer rate and cumulative herbage yield ($p = 0.0002$) was found. In 2012, the urine treatment significantly affected cumulative herbage yield ($p < 0.0001$). In 2011, cumulative herbage yield increased with total nitrogen inputs of up to 675 kg N ha^{-1} compared with 435 kg N ha^{-1} in 2012. N_2O emissions were recorded biweekly from March to October using the Hutchinson and Mosier (1981) vented chamber method in 2011 and 2012. Findings revealed a significant

interaction between urine treatment x fertilizer rate interaction and cumulative seasonal flux ($p = 0.0061$) in 2011 and the urine treatment ($p < 0.0001$) in 2012. There was a significant exponential relationship between fertilizer rate and cumulative seasonal flux in respect of urine treatment in 2011 ($p<0.0001$) and 2012 ($p<0.0001$). The range of % applied N lost through N_2O was between 0.518-1.781% for treatments in 2011 and 0.126-0.395% in 2012. The research supports the IPCC recommendations of 1.25% +/- 1% applied N lost as N_2O.

ACKNOWLEDGEMENTS

Two and a half years ago I began a journey. Little did I know, this journey would not only be to finish my Master of Science degree but also to develop my sense of character, challenge my independence, teach me countless new things, and provide a means to meet extraordinary people.

I want to thank my advisor John Guretzky for giving me this opportunity to study, teach, and do research under him at the University of Nebraska-Lincoln. His guidance allowed me to pursue multiple disciplines within upper education and work on a variety of research projects domestically and abroad.

When I started this journey, I never dreamed of learning the ins and outs of greenhouse gas sampling but with exceptional committee members I was given the support and guidance to keep my head up and continue moving along. I am truly thankful to have met Virginia Jin and even more thankful that she agreed to sit on my committee. She has provided lab and funding support for this project as well as given outstanding advice throughout my graduate program. Rhae Drijber and Martha Mamo also provided essential advice throughout my program and were great role models as female scientists and experts.

I want to thank everyone from UNL range and forage and USDA that assisted in collecting data, analyzing data, and supporting me in my project. Chris Barber was the best technician anyone could ask for and with the assistance of Dave Guretzky we applied ruminant urine better than anyone I know. Jon Soper, Ben Schiltz, Lars Anderson, Ellen Miller, and Derek Mettenbrink all had the opportunity to capture trace gasses with me or clip grass with scissors and I am very thankful for their assistance.

Susan Siragusa, Ryan McGhee, Carla Ahlschwede, and the entire USDA-ARS staff made me feel welcome and helped me in every way they could while I worked in their lab and learned from the greenhouse gas experts.

I would have never known about the Sandhills or had any interest in range and forage science if it were not for Lars Anderson and Jess Milby drawing a map of Nebraska for me while we were in New Zealand together in 2008. From there, Walter Schacht took me under his wing and with his guidance I have been a range scientist ever since. Walt and Carol Schacht have been my Lincoln parents for nearly five years and I thank them for all of their support and advice. Cheryl Dunn has been a supporter of mine since the beginning and with her amazing listening skills and unending advice; I am graduating a more confident and better person.

Last but not least, I want to thank my family and my bible study. My parents have always been supportive of my dreams and aspirations and with their love and prayers I have become the woman I am today. My brothers and my extended family have become increasingly essential in the past semester and I want to thank them for putting up with me and entertaining my late night calls. The women in my bible study and my church family were an integral support system of prayers and support which was never ending. Thank you to everyone who made this project a success, I could not have done it without you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

Chapter 1

A Review of Current Literature

1.1 INTRODUCTION

Greenhouse gases (GHG) can be defined as any gas that contributes to increasing atmospheric temperatures (i.e. the "greenhouse effect") by absorbing infrared radiation. These gases help to regulate temperature within the Earth's atmosphere and keep it at a level that supports life (Moss 2000). Atmospheric gas concentrations increase as the net result of both natural and anthropogenic emission and consumption processes. The amount of infrared radiation held in the atmosphere is correlated with the temperature of the Earth (Yunshe et al. 2000; IPCC 2007). Therefore both natural and anthropogenic processes involved in GHG dynamics are intimately linked to climate change.

Greenhouse gas emissions are formed through natural and man-made processes and through a variety of enterprises. This study focuses on nitrous oxide (N_2O) emissions in managed pasture ecosystems consisting of smooth bromegrass (*Bromus inermis* Leyss.). Livestock plays a major role in nitrogen cycling processes in managed pastures by affecting plant growth and nutrient availability. This chapter provides a review of current literature pertaining to U.S. agricultural GHG emissions, as well as a review of animal excretion components, nitrogen fertilizer effects, and growth and development of smooth bromegrass.

1.2 SOURCES AND DEVELOPMENT OF NITROUS OXIDE

The major naturally-occurring GHGs are water vapor, CO_2, N_2O, CH_4, and ozone (O_3) (Albert et al. 2011). Greenhouse gas emissions are typically reported as carbon dioxide equivalents (CO_2e), which provides a standardized metric to quantify and

compare various GHGs based on their ability to capture infrared radiation, or global warming potential (GWP). One CO_2e of a non-CO_2 GHG represents the equivalent, time-integrated radiative forcing from one molecule of CO_2 over a given time horizon. Radiative forcing is the net radiative flux change induced at the tropopause assuming there is no change in stratospheric temperature (IPCC 1990). For example, one molecule of N_2O has a radiative forcing potential that is equivalent to 310 molecules of CO_2 over a 100 year time frame; thus, N_2O has a GWP of 310 (IPCC 1995). The total emission of a given non-CO_2 GHG expressed in CO_2e, therefore, is equal to the total emission of the non-CO_2 GHG multiplied by its global warming potential (IPCC 2007).

Nitrous oxide has greater radiative forcing than CO_2 because it has absorption lines in the "spectral window" whereas CO_2 and H_2O are weak and with the exception of O_3, absorption is virtually without competition (Adviento-Borbe 2005). Nitrous oxide can diffuse through the troposphere to the stratosphere where it is lost to photolysis and other processes. Once in the stratosphere it can be globally circulated due to its long residence time (nearly 100 years). The ability for N_2O to exist in both troposphere and stratosphere allows it to contribute to tropospheric warming and stratospheric ozone depletion (National Academy of Sciences 2003).

1.3 THE ROLE OF AGRICULTURE IN U.S. GHG EMISSIONS

In the United States, agriculture is the fourth largest contributor to GHG emissions (Albert et al. 2011). This includes emissions from managed grasslands and rangelands. In grassland and rangeland ecosystems, carbon dioxide (CO_2) is cycled among living and dead plant matter, soil microorganisms, and the atmosphere and may be stored within soil to make this ecosystem a carbon sink. Grasslands also may serve as a

source for CO_2 particularly during drought and after intensive defoliation when plant and soil respiration exceeds CO_2 fixation through photosynthesis. Increasing CO_2 storage in grasslands through soil organic matter accumulation has been identified as a strategy to mitigate climate change. Another important carbon-based GHG in grassland and rangeland ecosystem is methane (CH_4), which is an enteric fermentation product released into the atmosphere by grazing livestock and also can be exchanged with the soil (Soussana et al. 2004).

Although agricultural activities were responsible for 6.3% of total GHG emissions in the United States in 2009 (Albert et al. 2011), agriculture is the number one producer of N_2O emissions. Agricultural soils were responsible for nearly 70% of N_2O emissions in the United States in 2009 (Albert et al. 2011). Most agricultural emissions are from agricultural soil management, manure management, and field burning of agricultural residues (Albert et al. 2011). Nitrous oxide has increased by 18% in the atmosphere since the industrial era, but annual emissions of N_2O fluctuate year to year with no way to predict the upcoming year due to sensitivity caused by the amount of nitrogen (N) fertilizer applied, weather patterns, and crop type.

Up to 10% of the atmospheric N_2 annually fixed by commercial conversion to N fertilizer becomes nitrous or nitric oxides (N_2O, NO), which are released during nitrification and denitrification processes (National Academy of Sciences 2003, Hopkins 2004, Smith 2010). Figure 1.1 from Baggs 2008, demonstrates how N_2O can result from nitrification, nitrate ammonification, and denitrification. These processes occur simultaneously and can compete for products depending on the environmental conditions. Nitrification is one component of mineralization, the process of oxidizing inorganic

ammonia into nitrite and nitrate (Eq. 1.1). This process is facilitated by ammonia-oxidizing bacteria including the genera *Nitrosomonas, Nitrospira, Nitrococcus,* and *Nitrosolobus*. Emissions of NO and N_2O occur naturally during the enzymatically-driven conversion of ammonium to nitrite and nitrate as by-products (Eq. 1.2). Adverse temperature or moisture conditions, however, can increase inefficiencies during N transformations that result in greater release of NO and N_2O. Nitrification may be influenced by soil pH and O_2 concentrations, but it does not seem to be affected by carbon (C) additions because nitrifiers are more ammonia limited compared to energy limited (Smith 2010).

Denitrification is the predominant source of N_2O and was thought to be the sole source until 1980 (Smith 2010). Emissions of N_2O develop through the anaerobic biological reduction of nitrate or nitrite to gaseous forms of N (Equation 1.2), especially in high moisture and fertilized conditions. Enzymes including nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase catalyze this reaction and the transport of electrons is fueled by ATP (Smith 2010). This process allows inorganic oxidized N compounds in the soil to return to the atmospheric N_2 pool (de Klein et al. 2003). When oxygen becomes available, however, it can easily bind to N_2 to become NO or N_2O. Many microbial groups have the ability to denitrify under different situations and may be able to switch preferences under certain conditions (Smith 2010). The rate of denitrifcation is controlled by C and N availability, O_2 concentrations, pH, and temperature.

1.4 N_2O EMISSIONS FROM MANAGED PASTURES

Pasture management interacts with naturally occurring soil processes that influence the production of N_2O in grasslands. Management factors include fertilizer applications and grazing practices which supply nutrient inputs from commercial N and animal excretions, respectively. The interaction of management with environmental conditions such as temperature and moisture availability influence pasture productivity and resulting C and N concentrations in soil and dead plant matter.

1.4.1 Nitrogen Fertilizer

Grazing management, fertilizer application, and reclamation of land from grassland to agricultural use and vice versa are critical factors affecting N_2O development in the soil (Yunshe et al. 2000). Managed grasslands are typically fertilized to increase production which causes increases in N_2O emissions that are larger than found in natural ecosystems (Soussana 2007). Studies have shown that losses of fertilizer N as N_2O are affected by fertilizer type, amount of fertilizer, method and timing of application, and vegetation or crop type (Adviento-Borbe 2005). Losses have been shown to be as low as 0.01% of fertilizer N applied as calcium nitrate or sodium nitrate and as high as 6% in a study using anhydrous ammonia (Eicher 1990, Adviento-Borbe 2005). Urea and anhydrous ammonium forms of fertilizer have been shown to have higher amounts of N_2O emissions compared to controlled-release N fertilizers coated with polyolefin or calcium-bound fertilizers (Adviento-Borbe 2005).

A two year study in England found that N_2O emissions in unfertilized grasslands were consistently less than 5 g ha^{-1} day^{-1} and emissions from fertilized plots were concentrated for about 3 weeks after fertilizer application. They also found that losses

from urea are highly associated with high water-filled pore space causing losses to be higher during wet seasons of the year. Loss of N through N_2O for urea fertilized grassland was 0.8% of applied in N in 1992 and 1.4% in 1993 in this study (Clayton et al. 1997).

1.4.2 Animal Excretion

Animal excretion, particularly urine, has been found to significantly increase N_2O emission rates (de Klein and Logtestijn 1994, de Klein et al. 2003). In the United States, animal excreta contribute 25% of the anthropogenic sources of N_2O (National Academy of Sciences 2003). The loss of N to the atmosphere has been shown to reach 18% of the total N content of urine, equivalent to 20 to 50 kg N ha^{-1} y^{-1} made unavailable to plants (de Klein and Logtestijn 1994). High N_2O emissions correspond with high soil moisture and rainfall events. In addition, compaction, and soil pH play a large role in emission rates (National Academy of Sciences 2003). Compaction and soil pH can be affected at a microsite level by grazing animals as they graze and produce excrement.

Grasslands that are managed and grazed intensively receive a large amount of N returned to the soil as livestock urine. This amount totals 250 - 300 kg N ha^{-1} y^{-1} distributed across the landscape. Deposition rates within urine patches themselves, however, are much higher, ranging from 30 - 60 g m^{-2} (or 300 - 600 kg ha^{-1}) for each urination event (de Klein and Logtestijn 1994). On average, a typical cow urinates at a rate of 10 L m^{-2} (de Klein et al. 2003). The average defecation and urination data from 20 studies of dairy and beef cows and steers shows that the average animal defecates 10.9 times per day, covering an area of 0.05 m^2 per defecation (or 0.55 m^2 d^{-1}), and urinates 8.5 times per day, covering approximately 0.28 m^2 (or 2.38 m^2 d^{-1}) (Haynes and

Williams 1993). Knowledge about the difference in N_2O emissions in animal excretion areas along with the percentage of land occupied by urine spots would result in better estimations of N_2O emissions from a pasture.

Research compiled on urine application to pastures show that there are significant increases in N_2O emissions from areas treated with cow urine compared to untreated areas (Klein et al. 2003, Klein and Logtestijn 1994). The N deposited as urine is easily lost in gas form and gaseous N losses have accounted from 20 - 40% of total N applied through urine. A majority of this N has been shown to be lost as N_2O and N_2 gas (de Klein and Logtestijn 1994). Overall, research on N_2O emissions in pastures using chamber based methods do not differentiate between urine spots and non-urine spots, which could result in low estimates of actual N_2O fluxes in pastoral systems. Research using the eddy covariance method can result in N_2O emissions that more accurately portray the emissions occurring across the entire field.

1.4.3 Environmental Factors

In a grassland ecosystem, the nature, frequency and intensity of disturbances play a large role in the carbon balance and therefore GHG flux (Soussana 2007). Natural phenomena including temperature at sampling and moisture availability in the soil affect the measured gas flux. The percentage of water-filled soil pore space in the soil is closely related to soil microbial activity and has been linked to soil N_2O production (Linn and Doran 1984). Water-filled pore space is generally higher in no-tillage agriculture and would also be higher in grassland ecosystems due to the increased soil structure in these areas therefore resulting in more microbial activity over a given time period and higher N_2O fluxes.

1.5 MEASUREMENT OF N_2O FLUX

Methods to measure GHG fluxes in ecosystems range widely in scale and temporal frequency. Large-scale eddy covariance techniques integrate continuously measured fluxes over an entire ecosystem, but are expensive and limit experimental manipulations to large areas. In contrast, static chambers are relatively less expensive, but allow the measurement of many experimental treatments in close proximity to each other (Livingston and Hutchinson 1995).

The USDA Agricultural Research Service (ARS) has established widely used, chamber-based sampling protocols for the agency's cross-location Greenhouse gas Reduction through Agricultural Carbon Enhancement network (GRACEnet). The most recently published sampling protocols outlined several factors affecting variability in sample measurements, including: soil disturbance, temperature, humidity, pressure changes, gas mixing, chamber placement, frequency of sampling, and spatial variability (Parkin and Venterea 2010). Each factor, when addressed in a manner appropriate to site and research objective, can improve gas flux measurements by decreasing variability in manual sampling.

Soil disturbances during the installation and retention of anchors can have a significant effect on the gas flux measured at a particular site. Microclimate changes including shading, humidity, temperature, and water retention can all occur. Compaction of soil around the anchor can also impact flux measurements due to changes in physical soil properties which affect microbial activity and water movement. In some cases, the anchor can promote flooding within the installed area during heavy precipitation and cause high humidity and even algal growth on the soil surface. If changes in soil

microclimate effects are observed, the chamber should be moved to minimize collar height, alleviate flooding, and allow anchors to equalize in the soil following installation disturbance for at least 24 hours prior to sampling.

Changes in temperature due to shading caused by the anchor, heating of the anchor and chamber, or variation in sampling time can cause variability in gas measurements. Temperature has an effect on biological activity and on gas properties of expansion and contraction; therefore, the temperature within the chamber should be similar to the temperature outside the chamber. By using insulation or reflective material to line the chamber, a constant temperature can be better maintained. Keeping the sampling time short and installing a thermometer to track temperature changes can also be advantageous.

Natural pressure perturbations can be altered when using a closed chamber approach to gas sampling. In order to decrease the effect this change has on the movement of gas near the soil surface, the proper installation of vents in the chamber hood is recommended. This can be especially important when sampling is occurring in open areas prone to wind.

Diffusion and mixing of gases is rapid when sampling from bare soil, but with the addition of vegetation within the chamber, homogeneous mixing can be disrupted. The use of a manifold is highly recommended to extract gas from a variety of points in a sampling chamber. Although the placement of a small fan within the chamber is not advised due to pressure perturbations, pressure changes can be minimized with short uses of the internal fan and short chamber deployments if additional gas mixing is warranted.

Chamber placement is an important factor to consider when sampling N_2O. One of the goals of gas emissions sampling is to collect samples representative of ecosystem emissions. This cannot be done without at least some inclusion of vegetation in chambers. Some research actually states that N_2O emissions may be facilitated by living plants (Smart and Bloom 2001) although it can complicate the interpretation of CO_2 flux data. The inclusion of vegetation must be carefully considered since increases in chamber height and volume can decrease flux detection sensitivity. Although some situations may require the movement of chambers seasonally or yearly, in grasslands have shown no apparent negative effects when installed for over 10 years (Parkin and Venterea 2010). Sampling frequency is also an important part of accurately measuring N_2O emissions and calculating cumulative fluxes. Sampling weekly can provide losses in fluxes of 14%-20% (Smith and Dobbie 2001, Parkin 2008) whereas sampling every 14 days can provide losses of 50% and sampling each 21 days can provide losses of up to 95% (Parkin 2008).

Gas fluxes are measured by finding the rate of change in gas concentrations in the chamber headspace. Chamber deployment of 30 - 60 minutes and use of at least 3 time points can decrease bias. Gases are best removed from the chamber using a syringe removing 5 to 30 ml of gas and then injected into an evacuated glass vial. There are many types of vials and septa that can be used in conjunction with gas flux sampling. Exetainer vials from Labco maintained > 90% of the overpressure for 13 days and had low variability when punctured 5 times with a 22 gauge needle (Parkin and Venterea 2010). Samples should be processed as quickly as possible.

Gas analysis for N_2O is performed by gas chromatography in the form of electron capture detection. Samples should be run in sequence with standards run periodically to minimize error. After the gas samples are run through gas chromatography, there is no best method for data analysis but several methods are suitable and appropriate for flux calculations. Gas samples are plotted on a graph of gas concentration verse time. A regression line is then fit to the graphed points. The slope of the line is then multiplied by the chamber volume and divided by the chamber surface area to result in flux per area per time.

This rate of change may be linear regression but it may also be a different relationship. The curvi-linear approach to regression can adapt fluxes that have resulted from a buildup of analyte concentrations in the chamber headspace resulting in an alteration of the diffusion gradient, non-vertical movement of gas in the soil, or leakage of gas from the chamber (Hutchinson and Mosier 1981, Livingston and Mosier 1995, Stolk et al 2009). A quadratic model has also been used to increase fluxes 10 - 40% compared to the linear regression model (Wagner et al. 1997). These methods were tested against each other using statistical analysis of the mean square error. At fluxes below 22 ug N m^{-2} h^{-2} the linear approach has the lowest mean square error although other characteristics such as curvi-linearity and analytical precision need to be taken into account (Parkin and Venterea 2010). After taking into account the sampling protocol suggestions an experimental design that is scientifically sound and encompasses the specific needs of measuringN_2O emissions in smooth bromegrass pasture was developed using a variety of methods.

1.6 HISTORY OF SMOOTH BROMEGRASS

Historically, smooth bromegrass has been used across Europe, Asia, and North America as a highly productive forage crop for hundreds of years. During the drought of the 1930's, smooth bromegrass was found to have much better drought tolerance then Kentucky bluegrass (*Poa pratensis*), and therefore, interest of its use in the United States began to grow. Since then, smooth bromegrass has been deemed the most important and widely grown bromegrass and one of the more productive, nutritious, and palatable forages in the Great Plains (Wheeler 1950, Newell 1973, and Vogel et al. 1996). Smooth bromegrass has been utilized in pastures for grazing and haying along with stabilizing road sides, ditches, and mine tailings across the United States and Canada (Otfinowski et al. 2006).

Smooth bromegrass was introduced from Hungary to the California Agricultural Experiment Station between 1880 and 1884 (Wheeler 1950, Engel 1983, Vogel et al. 1996, Otfinowski et al. 2006, Salesman and Thomsen 2011). Packets of Hungarian origin seeds were given to farmers starting in 1884 for trial plantings and in 1889 and 1896 smooth bromegrass seeds from Russia were distributed to 43 states. The bromegrass originating from Russia adapted well to the Northern Great Plains, whereas bromegrass from Hungary was found to favor Nebraska, Kansas, Iowa, and Missouri latitudes (Wheeler 1950).

Smooth bromegrass is used primarily for pasture, hay, or soil conservation. It can out-produce almost all other cool-season grasses, and it does not have alkaloid or other anti-quality issues like reed canarygrass (*Phalaris arundinacea)* (Vogel et al. 1996). It is very palatable making it excellent for livestock and wildlife especially during the vegetative stage (Stubbendieck 2011). Smooth bromegrass is estimated to cover several

million hectares of pasture in the North America (Vogel et al. 1996). Production can be limited by drought, heat, or cold stress, but overall smooth bromegrass is more drought tolerant then most cool-season grasses, becomes semi dormant in the summer, and persists in cold ecotypes across Eurasia and North America. Smooth bromegrass is also moderately tolerant of saline soils.

1.6.1 Description and Growth

Smooth bromegrass is in the *Bromus* or bromegrass genus and differs from many of the other epithets due to its awnless lemmas. The name comes from *bromos*, the Greek word for oat referring to the panicle inflorescence or *broma*, the Greek word food and *inermis* meaning unarmed (Hitchcock 1971, Vogel et al. 1996). Smooth bromegrass is an erect grass standing 0.4-1.2 m tall and has prominently veined, closed sheath, and flat blades measuring 15-40 cm long and 4-15 mm wide. The blades are glabrous to pubescent with scabrous margins and contain a conspicuous "W" or "M" constriction. Smooth bromegrass has a panicle inflorescence that is 7-24 cm long and is narrow to somewhat open (Engel 1983, Stubbendieck et al. 2011).

Germination occurs early in the spring for this cool season grass in soil temperatures below 7°C and even under snow cover (Otfinowski et al. 2006). Growth is rapid in the spring starting in March and continues through early May with anthesis in June. Maximum yields have been reported in Nebraska as early as 25 May (Engel et al. 1987, Otfinowski et al. 2006). Often there is little to no growth of smooth bromegrass in the mid to late summer but growth resumes in the fall. Fall tillers emerge but do not elongate like spring tillers therefore protecting them from fall defoliation and storing

carbohydrates for the winter months (Engel 1983, Otfinowski et al 2006, Salesman and Thomsen 2011).

Smooth bromegrass spreads through aggressive rhizomes and large, light seeds that are easily carried by the wind (Newell 1973). New tillers arise from rhizomes and basal buds early in the season and productivity increases rapidly in spring but levels off and declines by late summer. Roots are concentrated in the upper 10 cm of soil although they may penetrate over 1.5 meters deep. After anthesis, seeds are spread through wind dispersal mostly within 3.5 meters of the source or through animal or insect transportation. Seeds have been found to retain more than 70% viability when stored for 6 years under cool dry conditions (Otfinowski et al. 2006).

Establishment of smooth bromegrass is best seeded by drill to allow more accurate control of seeding rate and depth, although broadcast methods may work in certain situations. In Nebraska, smooth bromegrass is planted for monoculture pasture at 11.2-16.8 kg PLS ha^{-1}. If it is planted with a companion species, this rate decreases. Stand success is best with fall plantings rather than spring plantings which can allow weed and companion competition (Newell 1973).

1.6.2 Fertility of Smooth Bromegrass

Soil fertility can be a major limiting factor to smooth bromegrass forage yields especially in old stands. Two to three years after establishment, smooth bromegrass pasture can become "sod-bound," resulting in decreasing yields due to poor nitrogen availability and the presence of few fertile tillers (Wheeler 1950, Newell 1973, Vogel et al. 1996, Otfinowski et al. 2006). Studies conducted on old established stands of smooth bromegrass found that this condition could be easily remedied with fertilizer application

but not with tillage practices including disking (Rehm 1971). The "sod-bound" condition may be explained by the thick layer of dead grass that is present in smooth bromegrass pastures. This thatch traps nutrients until decomposition can occur therefore not allowing the new plants access to nutrients. It has also been shown that this thatch layer can play a key role in seedling emergence of competing species there by creating a competitive edge for smooth bromegrass (Williams and Crone 2006).

Fertilizer needs are directly related to available moisture and growing season length. In eastern Nebraska, forage yields increased with fertilization up to 180 kg ha^{-1} although yields resulting from fertilizer application between 90 and 135 kg ha^{-1} were only slightly lower (Vogel et al. 1996). Wedin (1974) noted diminishing yields and low economic return occur for nitrogen rates exceeding 134 kg ha^{-1} for smooth bromegrass. Furthermore, Rehm (1971) found that dry matter production increased with rates of N up to 180 kg ha^{-1} and that no difference in yields resulted from comparing rates of 180 and 270 kg ha^{-1} N. Smooth bromegrass responds well to fertilizer applied in the fall or early spring and if fall moisture is sufficient, dividing yearly fertilizer allowance into two application may increase fall forage growth (Newell 1973)

1.6.3 Environmental Impacts

Herbivory from ungulates, birds, and insects can affect forage production. Being a very palatable grass, smooth bromegrass is eaten by livestock and native ungulates that inhabit the range where it grows. Its seeds are also readily eaten by deer mice (*Peromyscus maniculatus* Wagner) and other small mammals. Although smooth bromegrass is not often the first preference for deer (*Odocoileus virginianus* Zimmerman, *O. hemoides* Rafinesque) or elk (*Cervus elaphus* Linnaeus), it can provide important

winter forage options. Birds including Canada geese (*Branta canadensis* L.) and blue geese (*Chen caerulescens* L.) eat the vegetative plant parts whereas others like the grasshopper sparrow (*Ammodramus savannarum* Gmelin) use smooth bromegrass pasture for shelter and source of insects. Seed production of smooth bromegrass can be greatly reduced by seed midges (*Stenodiplosis bromicola* Marikovsky & Agafonova) and thrips (Thysanoptera: Terebrantia and Tubulifera) an d seedlings are susceptible to several species of cereal aphids (*Diuraphis noxia* Mordvilko, *Schizaphis graminum* Rondani, *Macrosiphum avenae* F., *Rhopalosiphum padi* L.)(Newell 1973, Otfinowski et al. 2006). Planthoppers (*Prokelisia crocea*) and leafhoppers (*Endria inimical* Say, *Doratura stylata* Boheman, *Psammotettix alienus* Dahlbom) are also very common in smooth bromegrass pastures feeding on vegetative material (Otfinowski et al. 2006).

Besides herbivory, smooth bromegrass is also negatively affected by nematodes and diseases especially in moist soil environments. Root lesion nematodes including *Pratylenchus penetrans* (Cobb) and *P. neglectus* (Rensch) can cause detrimental damage to smooth bromegrass root systems. Leaves and culms can become infected with leaf blotches, rusts, scald, spots, and stripes due to the presence of fungi. This is particularly true in ecosystems with humid conditions (Newell 1973, Otfinowski et al. 2006). Smooth bromegrass in also susceptible to winter crown rot and snow molds although its tolerance is higher than most common forages. Lastly, the barley yellow dwarf virus and bromegrass mosaic virus can affect smooth bromegrass stands.

1.7 CONCLUSION

Greenhouse gases are extremely important to sustaining life on Earth but quantifying the increases of these gases in the past century has been difficult along many

lines. Scientists are just beginning to understand the complex relationship between gas fluxes in different ecosystems and the variables that affect the flux measurements. More research needs to be done to completely understand the implications of added GHG to our atmosphere and the role that natural environments like grasslands play in regulating those gases.

This study was concerned with GHG emissions in managed pasture ecosystems with a goal to understand how soil GHG production is influenced by nitrogen fertilizer, animal excretion, and herbage removal. The objectives of this study were:

(i.) To determine nitrous oxide emissions in smooth bromegrass pasture managed with five rates of nitrogen fertilizer application and two animal excretion levels,

(ii.) To better understand the mechanisms controlling GHG emissions including soil moisture and soil temperature.

One field experiment over two field seasons was performed to achieve these objectives as well as laboratory measurements and calculations. The subsequent chapter describes the methods and results from the study.

LITERATURE CITED

Adviento-Borbe, Arlene A. (2005). *Understanding Soil Greenhouse Gas Fluxes in Intensive Maize-Based Cropping Systems*. Ph.D. Thesis. University of Nebraska: U.S.A.

Albert, C. M., Chen, P.-S., Anderson, M. E., Cain, M. E., Fishman, G. I., Narayan, S. M., Olgin, J. E., et al. (2011). *Draft-Inventory of US Greenhouse Gas Emission and Sinks 1990-2009*. *United Nations Framework Convention on Climate Change*.

Baggs, E. M. (2008). A review of stable isotope techniques for N_2O source partitioning in soils : recent progress , remaining challenges and future considerations. *Rapid Communications in Mass Spectrometry*, *22*, 1664–1672.

Clayton, H., McTaggart, I. P., Parker, J., Swan, L., & Smith, K. a. (1997). Nitrous oxide emissions from fertilized grassland: A 2-year study of the effects of N fertilizer form and environmental conditions. *Biology and Fertility of Soils*, *25*(3), 252–260.

de Klein, C. A. M., Barton, L., Sherlock, R. R., Li, Z., & Littlejohn, R. P. (2003). Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Australian Journal of Soil Research, 41*, 381-399.

de Klein, C. A. M., & van Logtestijn, R. S. P. (1994). Denitrification and N_20 emission from urine-affected grassland soil. *Plant and Soil, 163*, 235-242.

Engel, R. K., L. E. Moser, J. Stubbendieck, and S. R. Lowry. (1987). Yield accumulation, Leaf Area Index, and Light Interception of Smooth Bromegrass. Crop Science 27: 316-321.

Engel, Russell K. (1983). *Leaf Area Index, Light interception, and Growth of Smooth Brome.* M.S. Thesis. University of Nebraska: U.S.A.

Haynes, R. J. and P.H. Williams. (1993). Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. p. 119-199 Advances in Agronomy Vol. 49. Academic Press, San Diego, CA.

Hitchcock, A.S. and Agnes Chase. (1971). Manual of the Grasses of the United States. 2nd edition Mineola, New York.

Hopkins, William G, and Norman P. A. Huner. (2004). Introduction to Plant Physiology. 3rd edition Hoboken, New Jersey.

Hutchinson, G. L., & Mosier, A. R. (1981). Improved Soil Cover Method for Field Measurement of Nitrous Oxide Fluxes. *Soil Science Society of America Journal, 45*(2), 311-316.

Intergovernmental Panel on Climate Change. (2007). IPCC Fourth Assessment Report. World Meteorological Organization and United Nations Environmental Program.

Intergovernmental Panel on Climate Change. (1995). IPCC Second Assessment Report. World Meteorological Organization and United Nations Environmental Program.

Intergovernmental Panel on Climate Change. (1990). The IPCC Scientific Assessment. World Meteorological Organization and United Nations Environmental Program.

Kyoto Protocol. (1997). Kyoto Protocol to the United Nations Framework Convention on Climate Change. United Nations, Kyoto, Japan.

Linn, D. M., & Doran, J. W. (1982). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils 1, (1961), 1267–1272.

Livingston, G. P. and G. L. Hutchinson. (1995). Enclosure-based measurement of trace gas exchange: applications and sources of error. P. 14-51. P. A. Matson and R. C. Harriss. (ed.) Biogenic Trace Gases: Measuring Emissions from Soil and Water. Blackwell Science, Cambridge, Massachusetts.

Moss, Angela, Lean-Pierre Jouany, and John Newbold. (2000). Methane Production by Ruminants: its Contribution to Global Warming. Ann. Zootech. 49: 231-253.

National Academy of Sciences. (2003). Air Emissions From Animal Feeding Operations: Current Knowledge, Future Needs. The National Academies Press, Washington, D. C.

Newell, L. C. (1973). Smooth Bromegrass. p. 254-262. Heath, Maurice E. et al. (ed.) Forages. Iowa State University Press, Ames, IA.

Otfinowski, R., N. C. Kenkel, and P. M. Catling. (2006). The Biology of Canadian Weeds. 134. *Bromus inermis* Leyss. Canadian Journal of Plant Science, 87: 183-198.

Parkin, Timothy B. (2008) Effect of Sampling Frequency on Estimates of Cumulative Nitrous Oxide Emissions. Journal of Environmental Quality 37:1390-1395.

Parkin, Timothy B., and Rodney T. Venterea. (2010). Sampling Protocols. Chapter 3. Chamber-Based Trace Gas Flux Measurements. IN Sampling Protocols. R. F. Follett, editor. P. 3-1 to 3-39.

Rehm, G. W., W. J. Moline, E. J. Schwartz, R. S. Moomaw. (1971). The Effect of Fertilization and Management on the Production of Bromegrass In Northeast Nebraska. Research Bulletin – University of Nebraska—Lincoln. Agricultural Experiment Station, 247: 1-27.

Salesman, Jessica Bolwahn, and Meredith Thomsen. (2001). Smooth Brome (Bromus inermis) in Tallgrass Praires: A Review of Control Methods and Future Research Direction. Ecological Restoration 29: 374-381.

Stolk, P. C., Jacobs, C. M. J., Moors, E. J., Hensen, a., Velthof, G. L., & Kabat, P. (2009). Significant non-linearity in nitrous oxide chamber data and its effect on calculated annual emissions. *Biogeosciences Discussions*, *6*(1), 115-141.

Soussana, J. F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T. and Arrouays, D. (2004). Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management, 20: 219–230.

Soussana, J.F., and V. Allard et al. (2007). Full accounting of the greenhouse gas (CO_2, N_20, CH_4) budget of nine European grassland sites. Agriculture, ecosystems and environment, 121: 121-134.

Smart, D. R., & Bloom, a J. (2001). Wheat leaves emit nitrous oxide during nitrate assimilation. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 7875–8. doi:10.1073/pnas.131572798

Smith, K. A. and K. E. Dobbie. (2001). The impact of sampling frequency and sampling imes on chamber-based measurements of N_2O emissions from fertilized soils. Global Change Biology, 7:933-945.

Smith, Keith. (2010). Nitrous Oxide and Climate Change. London, England.

Stubbendieck, James, Stephan L. Hatch, and Neal M. Bryan. (2011) North American Wildland Plants. 2nd Edition Lincoln, Nebraska.

Vogel, K.P., K.J. Moore and L.E. Moser. (1996). Bromegrasses. p. 535–567. L.E. Moser. et al. (ed.) Cool-Season Forage Grasses. ASA, CSSA, and SSSA, Madison, WI.

Wagner, S. W., D. C. Reicosky, and R. S. Allessi. (1997). Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal. 89:279-284.

Wedin, W.F. (1974). "Fertilization of Cool-Season Grasses." Forage Fertilization. American Society of Agronomy, the Crop Science Society of America, and the Soil Science Society of America. P95-118.

Williams, Jennifer L., and Elizabeth E. Crone. (2006). The Impact of Invasive Grasses on the Population of *Anemone patens*, a Long-Lived Native Forb. Ecology, 87: 3200-3208.

Wheeler, W. A. (1950). Forage and Pasture Crops. New York, New York.

Yunshe, D., Shen, Z., Yuchun, Q. I., Zuozhong, C., & Yuanbo, G. (2000). Fluxes of CO_2, N_2O and CH_4 from a typical temperate grassland in Inner Mongolia and its daily variation. *Chinese Science Bulletin*, *45*(17), 1590–1594.

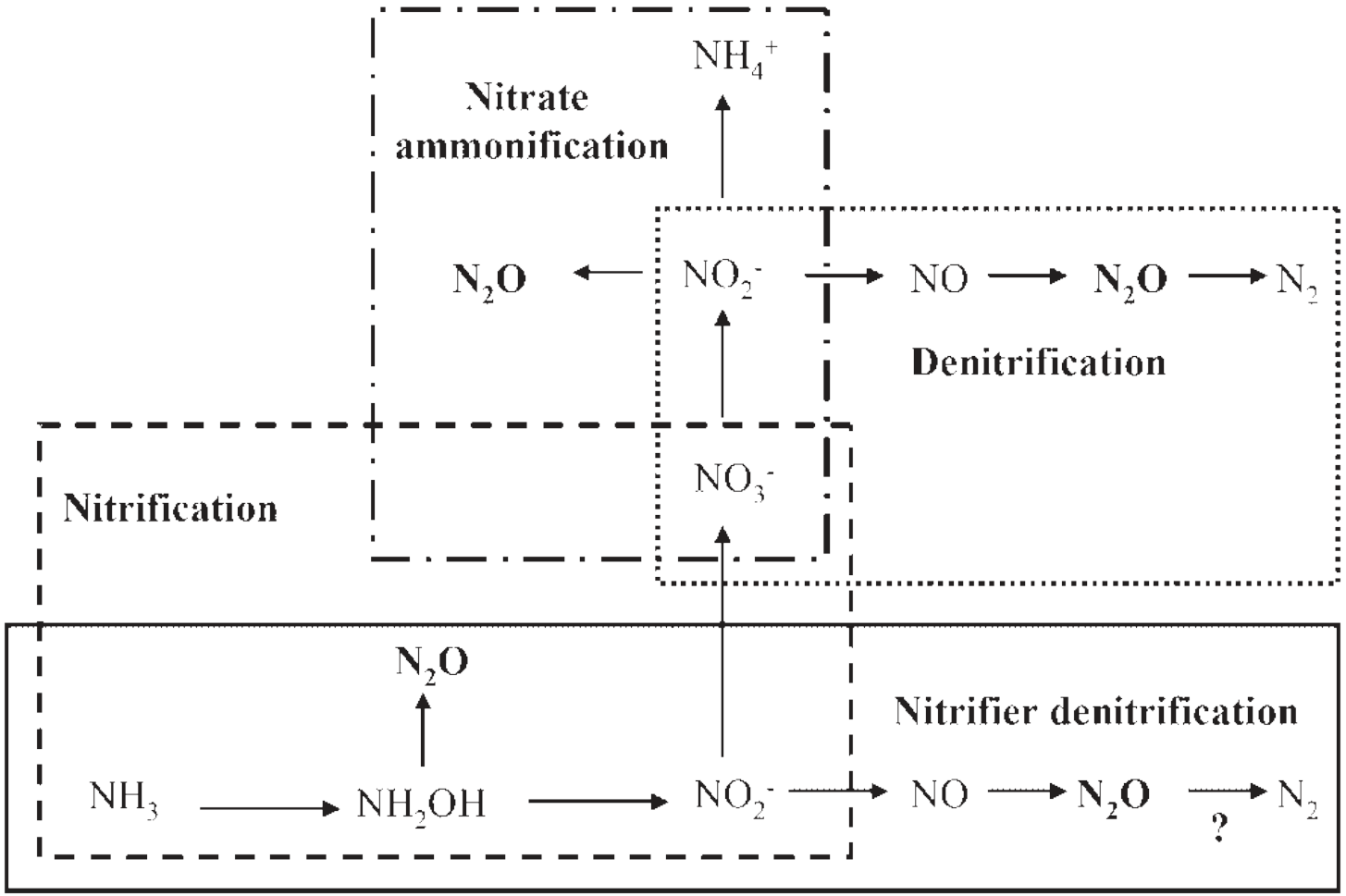
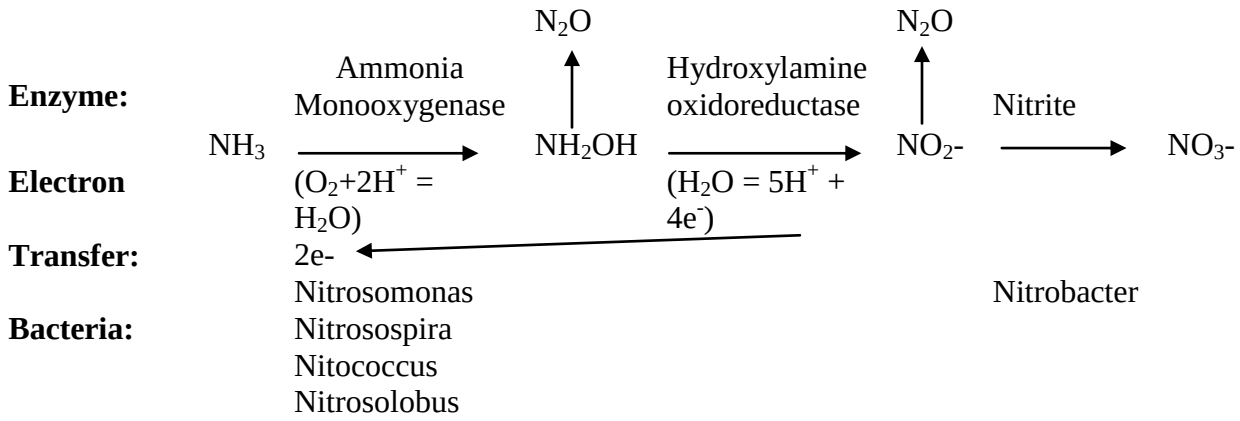
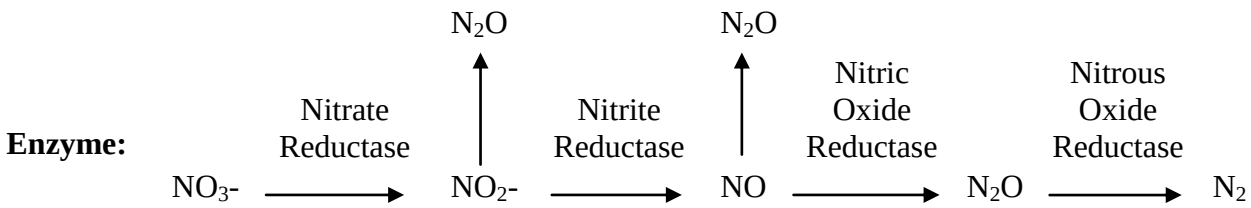


Figure 1.1 Microbial sources of N_2O in soil. Adapted from Baggs (2008) to show N_2O production from nitrification, nitrate ammonification, denitrification, and nitrifier denitrification.

Nitrification with Production of N_2O (Eq. 1.1)



Denitrification with Production of N_2O (Eq. 1.2)



Electron Transfer: $2\ NO_3^- + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$

Bacteria: Psedomonas spp.
Bacillus
spp.

CHAPTER 2

Nitrous Oxide Emissions from Smooth Bromegrass Pasture under Nitrogen Fertilizer and Ruminant Urine Application in Eastern Nebraska[1]

ABSTRACT

Nitrous oxide (N_2O) is a greenhouse gas primarily produced in soils by denitrifying and nitrifying organisms. In terms of global warming potential (GWP), N_2O has 310 times the GWP of carbon dioxide (CO_2). Agricultural soils account for 70% of emissions in the United States, but little data is available for contributions from managed pasture ecosystems. This study focused on the production of N_2O in smooth bromegrass (*Bromus inermis* Leyss.) pastures established on silt loam soils in eastern Nebraska. Thirty smooth bromegrass plots (1.5m x 1.5m) were treated with five different fertilizer treatments (0, 45, 90, 135, and 180 kg N/ha) and two urine treatments (urine and no urine). Herbage sampling was taken the day before sampling by clipping the grass within the anchor to a 10 cm stubble height and oven drying the samples. In 2011, a significant effect between the urine treatment x fertilizer rate and cumulative herbage yield ($p = 0.0002$) was found. In 2012, the urine treatment significantly affected cumulative herbage yield ($p < 0.0001$). In 2011, cumulative herbage yield increased with total nitrogen inputs of up to 675 kg N ha^{-1} compared with 435 kg N ha^{-1} in 2012. N_2O emissions were recorded biweekly from March to October using the Hutchinson and Mosier (1981) vented chamber method in 2011 and 2012. Findings revealed a significant interaction between urine treatment x fertilizer rate interaction and cumulative seasonal flux ($p = 0.0061$) in 2011 and the urine treatment ($p < 0.0001$) in 2012. There was a significant exponential relationship between fertilizer rate and cumulative seasonal flux

[1] Co-Authors: J. Guretzky, V. Jin, R. Drijber, M. Mamo.

in respect of urine treatment in 2011 ($p<0.0001$) and 2012 ($p<0.0001$). The range of % applied N lost through N_2O was between 0.518-1.781% for treatments in 2011 and 0.126-0.395% in 2012. The research supports the IPCC recommendations of 1.25% +/- 1% applied N lost as N_2O.

KEY WORDS carbon budgeting, global warming potential, denitrifying, nitrifying, *Bromus inermis* Leyss.

Agriculture is the number one producer of nitrous oxide (N_2O) emissions (Albert et al. 2011). Agricultural activities that influence N_2O production include, but are not limited to, livestock manure management, rice and other cereal crop cultivation, and agricultural soil management (Albert et al. 2011). These emissions not only decrease N availability to crops but contribute significantly to global warming since N_2O is 310 times as potent as carbon dioxide (CO_2) in capturing infrared radiation reflected from the earth's surface (IPCC 1995). As more infrared radiation is absorbed by N_2O molecules and other greenhouse gases in the atmosphere, atmospheric temperatures are predicted to increase.

Nitrous oxide emissions mainly occur as by-products of nitrification or through denitrification of N applied to agricultural soils. Nitrification and denitrification often occur simultaneously in the soil ecosystem and can compete for resources. Nitrification is the process of oxidizing inorganic ammonia into nitrite and nitrate which is facilitated by microbes in the soil (Smith 2010). Denitrification is the most common source of N_2O, which is a by-product of reducing nitrate or nitrite to dinitrogen gas (N_2). These processes are affected by environmental factors such as soil temperature, soil moisture,

and C and N availability in soil. Aerobic soil conditions favor nitrification whereas anaerobic soil conditions favor denitrification.

Anthropogenic factors such as grazing, fertilizer application, and reclamation are critical factors affecting N_2O development in the soil (Yunshe et al. 2000). Managed pasture can be fertilized to increase production, but this also increases N_2O emissions relative to unfertilized ecosystems (Soussana et al. 2007). Nitrous oxide losses from fertilizer are affected by fertilizer type, amount of fertilizer, method and timing of application, and vegetation or crop type (Ryden 1983, Eichner 1990, IPCC 1995, Clayton et al. 1997, Adviento-Borbe 2005). Losses have been shown to be as low as 0.01% of fertilizer N applied and as high as 6.8% (Eichner 1990). A two year study in England found that N_2O emissions in unfertilized grasslands were consistently less than 5 g N ha^{-1} d^{-1} but that emissions from fertilized plots were concentrated for three weeks after fertilizer application. Also in this study, loss of N through N_2O for urea fertilized pasture was 0.8% of applied in N in 1992 and 1.4% in 1993 (Clayton et al. 1997).

Studies have been completed in Europe and New Zealand on effects of fertilizer and urine inputs that provide a good basis for understanding of N_2O emissions in intensively managed pastures. A single cattle urination event can add 300-600 kg N ha^{-1} (de Klein and van Lotestijn 1994) to an area of 0.28 m^2 (Haynes and Williams 1993). Urine is excreted at a rate of 10 L m^{-2} (de Klein et al. 2003). In the United States, animal excreta contribute 25% of the anthropogenic sources of N_2O (National Academy of Sciences 2003). The loss of N to the atmosphere has been shown to reach 18% of the total N content of urine, accounting for 20 to 50 kg N ha^{-1} y^{-1} unavailable to plants (de Klein and van Lotestijn 1994).

Little research has been carried out on smooth bromegrass pasture to determine N_2O fluxes or on the affect that cattle urination have on fluxes in a typical management of pasture in eastern Nebraska. Nebraska's land area is 54% range, hayland, or pasture land (Stubbendieck and Kottas 2005) and beef production is its single largest industry ($12.1 billion revenue annually) (Nebraska Beef Council 2012) making this research important to producers and land managers across the state. Smooth bromegrass pasture is typically fertilized one or two times a year depending on moisture availability. The first application of fertilizer occurs in early spring at the start of vegetative growth and the second application occurs in the fall after air temperatures have fallen and plants are starting a second flush of growth. The suggested amount of nitrogen applied per year is between 90 kg ha^{-1} (Greenquist 2009) and 180 kg ha^{-1} (Rehm et al 1971) in eastern Nebraska, but can be as high as 300 kg ha^{-1} in other states (Zemenchik and Albrecht 2002).

The aim of this study was to investigate the effects of different fertilizer N rates and urine application on N_2O flux from smooth bromegrass pasture in eastern Nebraska. This research will fill a gap in current literature to provide baseline information for future research on GHG emissions in pastures of eastern Nebraska. The hypotheses are: (1) N_2O emissions will increase linearly with N fertilizer rate; and (2) urine application will double N_2O emissions compared to non-urine plots.

STUDY AREA

The experiment was initiated in 2011 at the Agriculture Research and Development Center near Mead, Nebraska (41° 6' N, 96° 30' W, 366 m above sea level). The average annual temperature was 10°C with a frost free period of 155-175 days per

year, and mean annual precipitation was 747 mm (1967-2011; High Plains Regional Climate Center 2012). The soil at the study site was a well-drained Tomek silt loam (Fine, smectitic, mesic Pachic Argiudoll) derived from loess, with 0-2% slope and high available water capacity. The ecological site description for this area was loamy upland (NRCS 2012). The pasture site had been in a smooth bromegrass monoculture for at least seventeen years prior to this experiment.

METHODS

Treatments

The experimental design was implemented in 2010 for data collection over the 2011 growing season. In the 2011 experiment, a 2594-m^2 study area was mowed to a height of 5 cm and divided into 30 plots during the summer of 2010. The plots were laid out on even ground and avoided areas with past dung excreta. One aluminum ring (65 cm in diameter and 20 cm in height) was installed to a 10-cm soil depth in the center of each treatment plot (1.25 m^2) (Fig. 2.1). Alleyways surrounding each plot were mowed at a 5-cm height to provide access. The design was repeated in February 2012 in an adjacent area to avoid residual treatment effects from the previous year's experiment.

For each year of the study, experimental treatments were assigned to plots in a completely randomized design (Fig. 2.1). Treatments consisted of five N fertilizer rates (0, 45, 90, 135, and 180 kg N ha^{-1}) as urea (46-0-0) and two urine application rates (no urine control and urine added). There were 10 treatment combinations total and three replications of each treatment. Treatments were randomly assigned to plots using a random number sequence and the fertilizer and urine treatments were applied uniformly to the entire plot area in both years. Nitrogen fertilizer was applied on 7 April 2011 and 3

April 2012 using 50 g of fine sand as a dispersant because of the small quantities of fertilizer applied. In 2011, urine was collected from domestic beef cattle (*Bos taurus)* owned by the University of Nebraska, Department of Animal Science and frozen daily in a 208 L drum over the month of April. The urine was thawed on 3 May 2011 and mixed thoroughly before application to plots on 4 May 2011. Before and during the urine collection period, the cattle were fed a diet consisting of smooth bromegrass hay with supplemented equivalent nitrogen content of smooth bromegrass pasture in spring. Supplementation was made by adding urea to the hay in a liquid form and co-feeding condensed distillers soluble (Table 2.1). This provided urine with a nitrogen content simulating the animals grazing smooth bromegrass in late April and early May. In 2012, urine was collected daily from domestic sheep (*Ovis aries)* owned by the University of Nebraska, Department of Animal Science, because of inavailability of cattle urine. Domestic sheep were fed the same diet as the cattle in 2011. Each sheep urine collection container was acidified with 100 ml of 0.9N sulfuric acid daily and emptied into a 208 L drum in the freezer each night over the month of March. Urine was placed in a refrigerator to thaw on 23 April 2012. It was mixed thoroughly and pH was adjusted to 7.0 with KOH before application to plots on 2 May 2012.

Urine treatments were applied manually with a watering can and spread evenly across each plot at a rate of 6.2 L m^{-2} during 2011 and 6.05 L m^{-2} during 2012. Control plots received the same volume of distilled water on the same days urine was applied. A sample of the urine from the 208 L drum was taken before and after application and tested for total N using a Costech Analytical ECS 4010. A difference of 3% in total N was found from the sample taken before application compared to after application. The

cattle urine from the 2011 experiment had a average total N content of 7.9 g N L^{-1} and the sheep urine from the 2012 experiment had 7.2 g N L^{-1} at the time of application. Nitrogen input rates from urine were 49 g N m^{-2} (490 kg N ha^{-1}) in the 2011 experiment and 43.5 g N m^{-2} (435 kg N ha^{-1}) in the 2012 experiment (Table 2.2).

N_2O AND HERBAGE SAMPLING

Measurement of greenhouse gas emissions followed the same procedure reported by Hutchinson and Mosier (1981). Gases were sampled on average every two weeks throughout the 2011 and 2012 growing season and every other day following urine and fertilizer application for a week. The 2011 growing season was 30 March to 18 October 2011, and the 2012 growing season was 29 March to 2 October 2012. Gas samples were taken in mid-morning between 08:00 and 12:00 hours. Vegetation inside the chamber was maintained at 10-cm height to ensure proper gas mixing and to simulate a continuous grazing situation. All biomass removed from within the ring area was oven dried at 60°C and weighed for herbage mass and yield determination. Grass outside the ring, but within the plot, was cut to the same height, and cuttings were deposited outside the study area to maintain consistency of stubble height and vegetation inputs in the plot areas. Herbage cuttings were taken one day before sampling throughout the growing season after a visual assessment showed that gases would be restricted in their flow when the hoods were put on the anchors. These cuttings simulate a continuously grazed pasture where grasses would be visited several times in a growing season.

Gas samples were taken by syringe using a stratified sampling design consisting of collecting gas at 10 minute increments for four time points (0, 10, 20, and 30 minutes). A 25 ml sample of gas was injected into an evacuated 12 ml Labco exetainer vial sealed

with a rubber septa (Labco Limited, High Wycombe, Buckinghamshire, England). Each vial septa was replaced after every other sampling (e.g. after six punctures, evacuation of sample vial, injection of 25 ml syringe sample and withdrawal for GC, times two) to prevent sample loss. Vials were transported to the laboratory in a lined tool box and stored at room temperature if analysis was completed within two days. Samples were run within seven days of sampling and kept in the refrigerator if sampling could not be completed within two days. Keeping samples in the refrigerator contracts the air in the vials and allows pressure to be taken off the septa. This is a precaution since the vials are certified to keep pressurized air for 13 days (Parkin and Venterea 2010). Analysis was conducted by gas chromatography on an automated Varian 450 GC (Bruker Daltonics, Fremont, CA, United States) equipped with an electron capture detector to quantify N_2O (Mosier et al. 2005). The machine was calibrated each week using a four point calibration method. The injection port septum on the Varian GC/MS was changed every 400 punctures.

N_2O FLUX CALCULATION

Nitrous oxide flux was calculated from the increase in concentration of N_2O in the chamber headspace with time (Livingston and Hutchinson 1995). Estimates of daily N_2O emissions between sampling days were made using linear interpolation between adjacent sampling dates (Halvorson et al. 2008). Cumulative fluxes were calculated by summing measured and linear interpolated daily fluxes over each growing season. Site baseline fluxes in 2011 and 2012 were calculated as the mean N_2O flux over all plots measured prior to any treatment applications each year.

Soil water availability, soil temperature, and air temperature were also measured on each sampling date. Soil volumetric water content was measured at a soil depth of 7.5-cm using a Field Scout TDR 100 soil dielectric constant probe (Spectrum Technologies, Plainfield, IL). The mean of three measurements was recorded, and adjusted using a soil-specific calibration. Soil temperature was measured once with an analog thermometer for each plot during the 30-minute gas sampling interval. Daily precipitation occurrences and the maximum and minimum air temperatures were gathered from the High Plains Regional Climate Center (HPRCC, Lincoln, NE). The weather station used to record these data was within two km of the experimental area.

STATISTICAL ANALYSES

Effects of N fertilizer rate, urine input, and their interactions on daily herbage harvest, seasonal herbage yield, daily N_2O fluxes and cumulative growing season emissions were examined with a mixed models repeated measures analysis of variance (SAS Institute, Cary, NC). Nitrogen fertilizer rate, urine input, and their interactions were considered fixed factors while sampling date was considered the repeated factor. Least squared means were determined for the daily herbage harvest and daily flux measurements using the mixed model procedure. Nonlinear regression procedures of proc nlin were used to examine the relationship between cumulative seasonal N_2O flux and N fertilizer rate for urine and non-urine treatments and relationships between measured soil variables and daily N_2O flux (SAS Institute, Cary, NC). Statistical comparisons were significant at the probability level of $\alpha=0.05$.

RESULTS

Weather

Precipitation during the 2011 growing season (March - October) was 699 mm compared to 386 mm in 2012 (Fig. 2.2). Growing season precipitation during the previous 44 years from 1968-2011 at the research site was 633 mm (High Plains Regional Climate Center, 2012). The annual and March - October temperatures for 2011 were 9.7°C and 15.9°C, respectively, both of which were comparable to the long-term, 44-year averages (10.0°C and 16.1°C, respectively). Saunders County, Nebraska was designated a disaster area due to severe drought conditions in the summer of 2012. The US Drought Monitor designated the research site as "abnormally dry" on 3 July 2012 and in "extreme drought" on 7 August 2012 (National Drought Mitigation Center, 2012). As a result, growing season temperatures in 2012 were higher than the 44-year average at 18.0°C (Fig. 2.3).

Herbage Production

2011

During 2011 growing season, biomass was collected every two weeks for a total of eight sampling events between May and September. Average harvested herbage biomass ranged from 1494 kg ha^{-1} on 23 May 2011 and 452 kg ha^{-1} on 2 May 2011 across all treatments. The cumulative herbage yield harvested averaged 8186 kg ha^{-1} across all treatments with the highest production occurring with application of 180 kg N ha^{-1} and urine and the lowest with application of 0 kg N ha^{-1} and distilled water as a control. The difference in average production from greatest to lowest yielding plots was 11,552 kg ha^{-1}.

The fertilizer rate × urine interaction significantly affected both the average sampling date harvest ($p = 0.0001$) and cumulative season harvest ($p = 0.0002$) (Table 2.3). Both incremental herbage mass and total seasonal herbage yield was lowest in the 0

kg N ha^{-1} fertilizer without urine treatment and highest in the 180 kg N ha^{-1} fertilizer with urine treatment. For no-urine treatments, total herbage yields did not differ between the 180 kg N ha^{-1} and 90 kg ha^{-1} fertilizer treatments. For urine-amended treatments, total herbage yields did not differ between the 135 kg N ha^{-1} fertilizer treatment compared to 90 kg N ha^{-1} and 45 kg N ha^{-1} fertilizer treatments. Cumulative herbage yield increased exponentially, both with the amount of total nitrogen input from urine N and fertilizer N (Fig. 2.4a) and with respect to each urine treatment (Fig. 2.4b).

2012

The 2012 growing season had severely limited water resources because of drought conditions. As a result, herbage was harvested on only three occasions in May, June, and August (Table 2.4). The highest daily harvest across all treatments occurred on 2 May 2012, and the lowest was on 28 August 2012 resulting in an average herbage harvest of 1256 kg ha^{-1} and 626 kg ha^{-1}, respectively. The cumulative herbage harvested in 2012 was on average 68% lower than in 2011. The 0 kg N ha^{-1} fertilizer without urine treatment was 77.8% lower in 2012 than 2011. The 0 kg N ha^{-1} fertilizer with urine treatment declined the least at 54.7% from 2011 to 2012. In the 2012 growing season, maximum herbage production occurred in the 180 kg N ha^{-1} fertilizer with urine treatment with 3778 kg ha^{-1}, and lowest production occurred in 0 kg N ha^{-1} fertilizer without urine treatment with 990 kg ha^{-1}.

The two-way interaction between urine input × date significantly affected herbage mass ($p < 0.0001$). No other main treatments or treatment interactions were significant. Smooth bromegrass with urine input produced 44.5% more plant biomass than smooth bromegrass without urine over the growing season. Urine treatments averaged 3631 kg ha^{-1} cumulative herbage yield where as distilled water treatments averaged 1614 kg ha^{-1}

cumulative herbage yield ($p < 0.0001$). Cumulative herbage yield response to fertilizer rates with distilled water was significantly lower than its corresponding fertilizer rate with urine. The addition of urine to plots with 0 kg N ha^{-1} resulted in 70.9% more cumulative herbage production than control plots without urine. Cumulative herbage yield increased exponentially with the amount of total N input from urine N and urea N (Fig. 2.5a). Treatments with urine showed no relationship between fertilizer application rate and cumulative herbage yield but treatments with urine showed an exponential rate of cumulative herbage yield increase with increase in fertilizer rate ($r^2 = .47$) (Fig. 2.5b).

Daily N_2O Fluxes

2011

Daily fluxes of N_2O in 2011 varied from non-detectable levels on several occasions to 713 g N ha^{-1} day^{-1} on 26 May 2011 from the 180 kg N ha^{-1} fertilizer with urine treatment. The highest daily emission rate from all treatments occurred on 26 May 2011 (Julian day 146) and was 20 to 300 times higher than daily fluxes measured on any other sampling date in 2011 (Fig. 2.6a and 2.6b). Daily fluxes in 2011 were significantly affected by the urine × fertilizer rate × date interaction ($p < 0.0001$). Statistical tests were re-analyzed, omitting day 146 fluxes, to test for treatment effects. Without day 146, the urine × fertilizer rate interaction was significant ($p = 0.0157$; Table 2.5).

2012

Daily fluxes were affected by a urine × date interaction ($p \leq 0.0001$) and the fertilizer rate × date interaction ($p = 0.01$). The urine × fertilizer × date interaction was approaching significance ($p = 0.0717$; Table 2.5). Average daily flux rates across all treatments in 2012 ranged from 13.2 g N ha^{-1} on 4 May 2012 to 0.14 g N ha^{-1} on 5 June 2012. The highest daily rate was 42.3 g N ha^{-1} from a plot with the 0 kg N ha^{-1} fertilizer

with urine treatment on 4 May 2012. Flux rates were not detected for several treatments and many sampling dates. The Fig. 2.7a shows the pattern of daily fluxes with the distilled water treatment and Fig. 2.7b shows the pattern of daily fluxes with the urine treatments through the 2012 growing season.

Soil Temperature and Moisture Effects on Daily N_2O Flux

Soil temperature varied from 3.8 to 27.5°C in 2011 and 12.8 to 26.7°C in 2012 (Fig. 2.8). There was no significant correlation between soil temperature and average daily flux for any treatment in 2011 or 2012 (Fig. 2.9). Soil moisture varied over the sampling season from 20.94 – 43.93% volumetric water content (VWC) in 2011 and 22.07 to 39.0% in 2012 (Fig. 2.10). Average daily N_2O flux responded as an increasing exponential function of VWC. In 2011, the trend was driven by the high fluxes on day 146 ($r^2 = 0.34$). When day 146 was removed from the analysis, the relationship strengthened ($r^2 = 0.42$). The exponential relationship was not statistically significant in 2011 with day 146 but when day 146 was removed, an exponential line was significant at $p = 0.0001$ ($y = 0.4229e^{0.0739x}$) (Fig. 2.11). In 2012, the exponential relationship with soil VWC explained a greater proportion of variance in daily N_2O flux ($r^2 = 0.62$) and was statistically significant at $p = 0.0125$ ($y = 0.0049e^{0.2002x}$) (Fig. 2.11).

Cumulative Growing Season N_2O Fluxes

2011

The magnitude of total growing season N_2O emissions varied between fertilizer and urine treatments, with a significant fertilizer rate × urine interaction ($p = 0.006$) (Fig. 2.12). The highest cumulative seasonal emissions were from the 180 kg N ha^{-1} fertilizer with urine treatment (12.1 kg N ha^{-1} $season^{-1}$), and the lowest emissions came from the 90

kg N ha^{-1} fertilizer without urine treatment (0.576 kg N ha^{-1} $season^{-1}$) (Table 2.6). Treatments with distilled water had consistently lower N_2O cumulative season fluxes measuring 0.57-1.9 kg N ha^{-1} $season^{-1}$. Total seasonal N_2O emissions in treatments with urine were 2.1-12.2 kg N ha^{-1} $season^{-1}$. The highest fertilizer rate (180 kg N ha^{-1}) had the highest emissions. Growing season N_2O emissions increased exponentially with fertilizer application rate in respect to urine treatment (Fig 2.13). Plots with urine had a significant exponential trend at $p = 0.0002$ and plots without urine had a significant trend at $p = <0.0001$. Each exponential line can be used to estimate the cumulative flux of plots with respect of urine application when fertilizer rate is known. The amount of N lost through N_2O emissions as a percentage of total N applied was between 0.64%-1.82% across all treatments, and was affected by a significant fertilizer rate × urine interaction ($p = 0.014$). Cumulative fluxes in 2011 were greatly affected by fluxes on day 146.

2012

Cumulative fluxes in 2012 were lower in distilled water treatments compared to urine treatments ($p \leq 0.0001$) (Table 2.7). Fluxes ranged from 0.118-1.09 kg N ha^{-1} $season^{-1}$ (Fig. 2.12). The highest and lowest fluxes came from the 180 kg N ha^{-1} fertilizer with urine and 0 kg N ha^{-1} fertilizer without urine treatments, respectively. Although the treatments with the highest and lowest added nitrogen were the treatments with the highest and lowest fluxes, the intermediate N treatment levels did not increase linearly as expected. For treatments without urine, there were no significant differences between any fertilizer level. In contrast, the urine-added treatments were all significantly different than the distilled water treatments, and showed a general increasing trend with fertilizer level. For urine-added treatments, cumulative N_2O emissions did not differ between the

0 kg N ha^{-1} and 45 kg N ha^{-1} fertilizer treatments and the 90 kg ha^{-1} and 135 kg N ha^{-1} fertilizer treatments. Growing season N_2O emissions in 2012 showed a similar trend as was seen in 2011 with regard to fertilizer rate, although the N_2O flux rates were much lower. Emissions in 2012 increased exponentially with added nitrogen fertilizer in regard to urine input (Fig. 2.14).

The highest percent loss of added N was observed in the 45 kg N ha^{-1} fertilizer without urine treatment (0.35%) and the 180 kg N ha^{-1} fertilizer without urine had the lowest percentage loss (0.11%). The fertilizer × urine interaction was significant at $p = 0.0002$ (Table 2.7). This interaction was driven by fertilizer rate. As fertilizer application rate increased, the percent of N lost as N_2O also increased.

DISCUSSION

Improving the understanding of vegetation and soil processes that cause N_2O fluxes to increase or decrease from agricultural ecosystems is of great importance. From a farming and ranching perspective, identification of how management impacts processes by which N_2O is emitted can help producers to be more nutrient use efficient and decrease emissions at the same time. In this study, we found urine input and N fertilizer application increased herbage production and N_2O emissions in smooth bromegrass pasture. Environmental factors including soil moisture also influenced N_2O emissions.

Cool season grass production in eastern Nebraska was above average in 2011 (USDA 2012), because of above average precipitation throughout the growing season under average temperature conditions (HPRCC 2012) and from the addition of fertilizer (Rehm et al. 1971, Vogel et al. 1996). Leaving a 10 cm stubble height, a cumulative average of 3.69-18.19 T DM ha^{-1} of smooth bromegrass forage was harvested across all treatments. As nitrogen input increased through urea fertilizer and urine input,

cumulative herbage production increased linearly. Contrary to our hypothesis, herbage production did not plateau with N fertilizer rate. This could be due to the above average soil moisture present during this year allowing the plants to take advantage of more nitrogen than a typical year. Herbage production may have been further enhanced if nitrogen applications were distributed more evenly throughout the growing season, which may have allowed more nitrogen to be used by the plants and decreased losses to leaching or N_2O (Clayton et al. 1997).

Lack of precipitation in 2012 affected herbage production greatly, resulting in one-third of herbage production seen in 2011. Among introduced cool-season forage grasses, smooth bromegrass is less drought tolerant than tall fescue (*Festuca arundinacea* Schreb.), but it is quite hardy and can survive in areas receiving as low as 280 mm of precipitation a year (Otfinowski et al. 2006). The lack of precipitation decreased the number of times stands were harvested from 8 times in 2011 to 3 times in 2012. Studies of smooth bromegrass show increasing forage yields with N fertilizer rates from 160 kg N ha^{-1} (Rehm et al. 1971) to 180 kg N ha^{-1} (Vogel et al. 1996) but a majority of herbage yield increases occur with fertilizer inputs only up to 90 kg N ha^{-1} (Vogel et al. 1996). That was not the case in 2012 of this study. Fertilizer rate was not significant, but the 0 kg N ha^{-1} fertilizer plots tended to produce less forage than all of the other fertilizer treatments. Urine input, however, was a significant factor to herbage production. Smooth bromegrass with urine produced 44.5% more forage yield than smooth bromegrass without urine over the growing season. Forage yield response to total N inputs from urine and fertilizer also showed an interesting pattern. Unlike 2011, forage yield reached a plateau when total N inputs exceeded 435 kg ha^{-1} in 2012. In this study,

significant increases in forage yield occurred with up to 670 kg N ha^{-1} in 2011 and 435 kg N ha^{-1} in 2012.

Daily N_2O fluxes varied greatly between days, but none were greater than fluxes on day 146 in 2011 compared to the rest of the 2011 season. This day greatly influenced the significance of treatment interactions. When data from this day were removed, only three treatment combinations were significant at $p \leq 0.100$. These treatment combinations are important to point out, but by taking out day 146, the daily N_2O data change since plots with flux peaks on the sampling date before or after day 146 became more significant. Using this point of thought, the 2011 daily fluxes were especially significant in all treatment combinations. The occurrence of one high day of fluxes raises concern about the timing of N_2O sampling since other fluxes could be missed and there is no good prediction of how long that high flux was occurring.

In 2012, no day influenced daily N_2O flux as significantly as day 146 in 2011. Even without an exceptional high flux peak, 2012 still showed a urine treatment effect at $p \leq 0.0001$ and a fertilizer rate effect at $p \leq 0.100$. The amount of nitrogen added to the plots with the urine treatment was more than typically added to smooth bromegrass pasture in eastern Nebraska, and thus nitrogen as well as moisture can play a role in N_2O fluxes since 2012 had very little moisture. The difference in fluxes between 2011 and 2012 demonstrate the importance of environmental factors such as precipitation and temperature. It also demonstrates why more research would need to be done to establish an appropriate seasonal flux for this ecosystem since year to year fluxes can vary so drastically. In 2011, the range of cumulative seasonal emissions were 0.38 -15.72 kg N ha^{-1} $season^{-1}$ compared to 0.08 - 1.45 kg N ha^{-1} $season^{-1}$ in 2012. Determining baseline

fluxes by calculating mean N_2O flux over all plots prior to any treatment applications each year likely led to overestimated non-growing season baseline fluxes because temperatures at this time were higher and soils had already experienced one or two warming events.

Daily N_2O fluxes increased exponentially with VWC. Although the exponential equation fit data from 2012 better than 2011, both years showed a trend that N_2O losses depended largely on volumetric water content. This was clearly shown by Linn and Doran (1984) both infield and in laboratory incubations. As volumetric water content increases, anaerobic activity would increase at an increasing rate, and therefore account for more N_2O emissions through denitrification. The correlation coefficients found in this study ($r^2 > .34$) were higher than reported by other authors (Ryden 1983, de Klein and van Lotestijn 1996, Clayton et al. 1997). Clayton et al. (1997) attributed low correlation coefficients to low mineral N especially in the winter sampling periods where soil moisture is high, temperature is low, and fluxes are low.

Urea fertilizer and urine or other organic slurries are typically higher producers of N_2O than other forms of fertilizer like anhydrous ammonia or ammonium nitrate (Eichner 1990). When comparing studies, many managed pasture operations have much more intense fertilizer management routines than used in our study which could explain why our values were on the lower end of average. High peak rates as seen on day 146 have occurred in studies where fertilizer application was in conjunction with precipitation events (Clayton et al. 1997, Ryden 1981). The high peaks on day 146 showed that we likely recorded a major N_2O emissions peak in 2011 but since this was absent in 2012, the peak may have been missed. Although we recorded a peak in 2011 there is no way to

determine how long the peak lasted or if the emissions measured were the highest from the plots. The lack of an N_2O peak in 2012 could have contributed to the low fluxes and low percentage of applied N lost across the season. Although looking at other studies can provide insight to interpretation of results, comparisons are difficult because N_2O emissions depend on nitrogen input, soil, crop type, and environmental conditions.

The patterns of emissions in 2011 varied in timing and quantity of cumulative emissions relative to 2012. A major peak occurred on day 146 in 2011 but no similar peak was observed in 2012. The highest peak in 2012 occurred on day 124. Changes in environmental conditions such as precipitation and temperature begin to explain these differences in fluxes and express the importance of long term studies for documentation of long-term average seasonal N_2O fluxes. This dissimilarity in concurrent years of data collection is not unique (Clayton et al. 1997) and environmental conditions from 2011 to 2012 in our study were more variable than other studies. Rainfall patterns (Clayton et al. 1997), temperature, organic C content, and oxygen availability (Eichner 1990), have all been cited as environmental factors that have influenced N_2O emission from one year to the next.

Using equation seven from Pleasants et al. (2007) the density of urine spots in a pasture can be determined from a pasture given the stocking rate (Eq. 2.1). This information coupled with the cumulative growing season emissions data from this study can give an appropriate pasture wide growing season flux estimate. Assuming a stocking rate of 5 animal unit months (AUM) per ha, 0.42 urinations per h, and each urination covering an area of 0.5 m^2, 4.67% of the pasture would be affected by at least one urination event. The stocking rate of 5 AUM was developed using the average cumulative

herbage yield from the 90 kg N fertilizer without urine treatment. This application could be useful to farmers or ranchers determining their N_2O emissions on a pasture scale or to government officials wanting to create N_2O inventories from pastures with grazing management.

In summary, our N loss rates support the Intergovernmental Panel on Climate Change (IPCC 2007) assessment while providing information for an ecosystem not readily studied in the past. The IPCC estimates that 1.25 ± 1% of N applied is lost through N_2O emissions (IPCC 2007). Herbage growth might continue to be stimulated during years of more than adequate rainfall with N rates larger than 80 - 120 kg N ha^{-1}, the recommended N input rate for this region (Kucera and Hancock 2006). In years of limited moisture, growth may plateau at high rates of N over 400 kg N ha^{-1}. The results on soil moisture and daily fluxes show that a linear relationship may not be the only way to interpret results and an exponential relationship may be more appropriate. Using an example from Linn and Doran (1984) daily flux values could be estimated from the exponential regression line. Data from 2011 and 2012 using non-linear regression may also provide a means for predicting cumulative N_2O fluxes in pastures given nitrogen fertilizer input in pastures with and without cattle urine. Determining the density of urine spots in a pasture and using N_2O emissions from this study may be useful to provide N_2O inventories on a pasture scale. Environmental factors influence N_2O fluxes immensely, and the importance of long term studies cannot be stressed enough. High peak fluxes raise concerns for future research implications and signal the need for eddy-covariance instrumentation that can track N_2O emissions daily if not hourly (Matson and Harriss 1995).

LITERATURE CITED

Adviento-Borbe, Arlene A. (2005). *Understanding Soil Greenhouse Gas Fluxes in Intensive Maize-Based Cropping Systems*. Ph.D. Thesis. University of Nebraska: U.S.A.

Albert, C. M., Chen, P.-S., Anderson, M. E., Cain, M. E., Fishman, G. I., Narayan, S. M., Olgin, J. E., et al. (2011). *Draft-Inventory of US Greenhouse Gas Emission and Sinks 1990-2009*. *United Nations Framework Convention on Climate Change*.

Clayton, H., McTaggart, I. P., Parker, J., Swan, L., & Smith, K. a. (1997). Nitrous oxide emissions from fertilised grassland: A 2-year study of the effects of N fertiliser form and environmental conditions. *Biology and Fertility of Soils*, *25*(3), 252–260.

de Klein, C. A. M., Barton, L., Sherlock, R. R., Li, Z., & Littlejohn, R. P. (2003). Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Australian Journal of Soil Research*, *41*, 381–399.

de Klein, C. A. M., & van Lotestijn, R. S. P. (1994). Denitrification and N_2O emission from urine-affected grassland soil. *Plant and Soil*, *163*, 235–242.

Eichner, M. J. (1990). N_2O from fertilized soils Eichner.PDF. *Journal of environmental quality*, *19*, 272–280.

Greenquist, M. a, Klopfenstein, T. J., Schacht, W. H., Erickson, G. E., Vander Pol, K. J., Luebbe, M. K., Brink, K. R., et al. (2009). Effects of nitrogen fertilization and dried distillers grains supplementation: forage use and performance of yearling steers. *Journal of animal science*, *87*(11), 3639–46.

Haynes RJ, Williams PH. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy, 49, 120-199.

Halvorson, Ardell D., Stephen J. Del Grosso, and Curtis A. Reule. (2008) Nitrogen, Tillage, and Crop Rotation Effects on Nitrous Oxide Emissions from Irrigated Cropping Systems. Journal of Environmental Quality 37: 1337–1344.

HPRCC High Plains Regional Climate Center. (2012). "Single Station Climate Summaries." http://climod.unl.edu/ Accessed 2 November 2012.

Intergovernmental Panel on Climate Change. (2007). IPCC Fourth Assessment Report. World Meteorological Organization and United Nations Environmental Program.

Intergovernmental Panel on Climate Change. (1995). IPCC Second Assessment Report. World Meteorological Organization and United Nations Environmental Program.

Kucera, M. J., & Hancock, R. (2006). Agronomy Technical Note No. NE-108. USDA-NRCS.

Linn, D. M., & Doran, J. W. (1984). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils 1. *Soil Science Society of America Journal*, *48*(1961), 1267–1272.

Livingston, G. P. and G. L. Hutchinson. (1995). Enclosure-based measurement of trace gas exchange: applications and sources of error. P. 14-51. P. A. Matson and R. C. Harriss. (ed.) Biogenic Trace Gases: Measuring Emissions from Soil and Water. Blackwell Science, Cambridge, Massachusetts.

Matson, P. A. and R. C. Harriss. *Biogenic trace gases: measuring emissions from soil and water*. Wiley-Blackwell.

Mosier A. R., A. D.Halvorson, G. A.Peterson, G. P. Robertson, and L. Sherrod. (2005). Measurement of net global warming potential in three agroecosystems. Nutrient Cycling in Agroecosystems 72: 67–76.

National Academy of Sciences. (2003) Air Emissions From Animal Feeding Operations: Current Knowledge, Future Needs. The National Academies Press, Washington, D. C.

National Drought Mitigation Center. (2012). "U.S. Drought Monitor." http://drought.unl.edu/ Accessed 2 November 2012.

Nebraska Beef Council. (2012). "State and National Facts." http://www.nebeef.org. Accessed 15 October 2012.

NRCS. (2012). Natural Resource Conservation Service Web Soil Survey. "Saunders County, Nebraska (NE155)." http://websoilsurvey.nrcs.usda.gov Accessed 28 October 2012.

Otfinowski, R., Kenkel, N. C., & Catling, P. M. (2006). The biology of Canadian weeds . 134 . Bromus inermis Leyss. *Canadian Journal of Plant Science*, *87*, 183–198.

Parkin, Timothy B., and Rodney T. Venterea. (2010). Sampling Protocols. Chapter 3. Chamber-Based Trace Gas Flux Measurements. IN Sampling Protocols. R. F. Follett, editor. P. 3-1 to 3-39.

Pleasants, A. B., Shorten, P. R., & Wake, G. C. (2006). The distribution of urine deposited on a pasture from grazing animals. *The Journal of Agricultural Science*, *145*(01), 81.

Rehm, G. W., Moline, W. J., Schwartz, E. J., & Moomaw, R. S. (1971). Effect of Fertilization and Management on the production of bromegrass in northeast Nebraska. *Research Bulletin-University of Nebraska Lincoln*, *247*, 1–27.

Ryden, J. C. (1981). Nitrous Oxide exchange between a grassland soil and the atmosphere. *Nature*, *292*, 235–237.

Ryden, J.C., (1983). Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium-nitrate. Journal of Soil Science 34, 355–365.

Smith, Keith. (2010). Nitrous Oxide and Climate Change. London, England.

Soussana, J., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., et al. (2007). Full accounting of the greenhouse gas (CO_2, N_2O, CH_4) budget of nine European grassland sites. *Agriculture, Ecosystems & Environment*, *121*(1-2), 121–134.

Stubbendieck, James and Kay L. Kottas. (2005). Common Grasses of Nebraska. University of Nebraska-Lincoln Extension, Lincoln, Nebraska.

USDA, National Agricultural Statistics Service. (2012). 2011 State Agricultural Overview. http://www.nass.usda.gov/Statistics_by_State/Ag_Overview/AgOverview_NE.pdf. Accessed 2 November 2012.

Vogel, K.P., K.J. Moore and L.E. Moser. (1996). Bromegrasses. p. 535–567. L.E. Moser. et al. (ed.) Cool-Season Forage Grasses. ASA, CSSA, and SSSA, Madison, WI.

Yunshe, D., Shen, Z., Yuchun, Q. I., Zuozhong, C., & Yuanbo, G. (2000). Fluxes of CO_2, N_2O and CH_4 from a typical temperate grassland in Inner Mongolia and its daily variation. *Chinese Science Bulletin*, *45*(17), 1590–1594.

Zemenchik, R. A., & Albrecht, K. A. (2002). Nitrogen Use Efficiency and Apparent Nitrogen Recovery of Kentucky Bluegrass, Smooth Bromegrass, and Orchardgrass. *Agronomy Journal*, *94*, 421–428.

Table 2.1 Percentages of feed components fed to cattle and sheep before and during urine collection in 2011 and 2012. Percentage of total dry matter is reported.

Feed Component	Percentage
Bromegrass Hay	82%
Condensed Distillers Solubles	10%
Urea	3%
Mineral Supplement	5%

Table 2.2 Urine N, fertilizer N, and total N from each treatment in 2011 and 2012.

Urine Treatment	N Fertilizer Rate	Urine N Applied		Total N Applied	
		2011	2012	2011	2012
	kg N ha^{-1}				
No Urine (DI only)	0	0	0	0	0
	45	0	0	45	45
	90	0	0	90	90
	135	0	0	135	135
	180	0	0	180	180
Urine	0	490	435	490	435
	45	490	435	535	480
	90	490	435	580	525
	135	490	435	625	570
	180	490	435	670	615

Table 2.3 Herbage production means and standard errors for 2011. Means in the same column with different letters are significantly different from each other. The urine x fertilizer rate interaction in 2011 was significant for both herbage sampling mass and cumulative herbage production.

Year	Urine Input	Urea N Fertilizer Rate	Herbage Sampling Mass	Cumulative Herbage Yield
	kg N ha^{-1}	kg N ha^{-1}	kg ha^{-1} d^{-1}	kg ha^{-1} yr^{-1}
2011	0	0	558.00[a]	4462.00[a]
		45	682.88[b]	5463.02[b]
		90	707.27[b]	5658.17[bc]
		135	877.85[c]	7022.76[cd]
		180	820.00[bc]	6558.00[c]
	490	0	939.66[c]	7517.25[d]
		45	1121.76[d]	8974.10[e]
		90	1319.28[e]	10554.27[f]
		135	1204.00[de]	9631.97[e]
		180	2001.77[f]	16014.16[g]
			p = .0001	p = .0002
		SE	+/- 143.00	+/- 903.79

Table 2.4 Herbage production means and standard errors for 2012. Means in the same column with different letters are significantly different from each other. In 2012, the urine treatment was significant but all other treatment affects and interactions were not statistically significant.

Year	Urine Input	Urea N Fertilizer Rate	Herbage Sampling Mass	Cumulative Herbage Yield
	kg N ha^{-1}	kg N ha^{-1}	kg ha^{-1} d^{-1}	kg ha^{-1} yr^{-1}
2012	0	0	456.97[a]	990.29[a]
		45	628.87[ab]	1685.18[bc]
		90	580.55[ab]	1348.71[b]
		135	756.58[b]	2131.61[cd]
		180	713.87[b]	1916.14[bd]
	435	0	985.24[c]	3406.07[ef]
		45	1170.10[c]	3635.00[ef]
		90	1411.18[d]	3957.31[e]
		135	1160.35[c]	3379.06[f]
		180	1818.03[e]	3777.85[ef]
		SE	+/- 197.88	+/- 552.96
2012 Means		0	721.10[a]	2198.18[a]
		45	899.48[b]	2660.09[b]
		90	995.86[b]	2653.01[b]
		135	958.46[b]	2755.34[b]
		180	1265.95[c]	2846.99[b]
		SE	+/- 139.88	+/- 391.20
	0		627.37[a]	1614.39[a]
	435		1308.98[b]	3631.06[b]
			$p < 0.0001$	$p < 0.0001$
		SE	+/- 88.47	+/- 247.34

Table 2.5 The statistical signficance of daily flux and treatment interactions for 2011 and 2012. 2011 data is run with and without day 146.

Effect	p
2011 Repeated Measure	
Urine	<.0001
Fert	0.0028
Urine*Fert	0.006
Date	<.0001
Urine*Date	<.0001
Fert*Date	<.0001
Urine*Fert*Date	<.0001
2011 (minus date 146)	
Urine	0.4598
Fert	0.0775
Urine*Fert	0.0157
Date	<.0001
Urine*Date	0.6513
Fert*Date	0.4379
Urine*Fert*Date	0.4287
2012 Repeated Measure	
Urine	<.0001
Fert	0.2811
Urine*Fert	0.3554
Date	<.0001
Urine*Date	<.0001
Fert*Date	0.0100
Urine*Fert*Date	0.0717

Table 2.6 Cumulative growing season N_2O fluxes for 2011 in average flux per season and % N lost as a function of applied N.

Treatment	Average Kg/ha/season	SE	% N lost	SE
Fert*Urine	p = 0.0061*		p = 0.0143*	
0 DI	0.809[a]	1.433	NA	0.362
45 DI	0.730[a]	1.433	1.622[ac]	0.362
90 DI	0.576[a]	1.433	0.640[a]	0.362
135 DI	1.640[a]	1.433	1.215[ac]	0.362
180 DI	1.906[a]	1.433	1.060[a]	0.362
0 Urine	3.343[c]	1.433	0.682[b]	0.362
45 Urine	2.089[ab]	1.433	0.390[c]	0.362
90 Urine	10.331[d]	1.433	1.781[b]	0.362
135 Urine	3.240[bc]	1.433	0.518[ac]	0.362
180 Urine	12.200[e]	1.433	1.821[d]	0.362
Means	p = 0.0029*		p = 0.0661	
0	2.076[ab]	1.013	0.341[a]	0.256
45	1.410[a]	1.013	1.006[bc]	0.256
90	5.453[c]	1.013	1.211[bd]	0.256
135	2.440[b]	1.013	0.867[c]	0.256
180	7.053[d]	1.013	1.440[d]	0.256
	p = <0.0001*		p = 0.5727	
DI Water	1.132[a]	0.641	0.907[a]	0.162
Urine	6.241[b]	0.641	1.039[a]	0.162

$p<0.05$ *Significant Interaction
Same letters in the same column represent no significant difference

Table 2.7 Cumulative growing season N_2O fluxes for 2012 in average flux per season and % N lost as a function of applied N.

2012 Data Treatment	Average Kg/ha/season	SE	% N lost	SE
Fert*Urine	p = 0.3553		p = 0.0002*	
0 DI	0.136[a]	0.132	NA	0.040
45 DI	0.178[a]	0.132	0.395[a]	0.040
90 DI	0.233[a]	0.132	0.259[b]	0.040
135 DI	0.265[a]	0.132	0.196[c]	0.040
180 DI	0.226[a]	0.132	0.126[d]	0.040
0 Urine	0.874[bd]	0.132	0.201[c]	0.040
45 Urine	0.689[c]	0.132	0.144[d]	0.040
90 Urine	0.915[b]	0.132	0.174[c]	0.040
135 Urine	0.766[cd]	0.132	0.134[d]	0.040
180 Urine	1.220[e]	0.132	0.198[c]	0.040
Means	p = 0.2810		p = 0.0060*	
0	0.505[ab]	0.093	0.100[a]	0.028
45	0.433[a]	0.093	0.269[b]	0.028
90	0.574[b]	0.093	0.217[c]	0.028
135	0.516[ab]	0.093	0.165[d]	0.028
180	0.723[c]	0.093	0.162[d]	0.028
	p = <0.0001*		p = 0.3415	
DI Water	0.208[a]	0.059	0.195[a]	0.018
Urine	0.893[b]	0.059	0.170[a]	0.018

p<0.05 *Significant Interaction
Same letters in the same column represent no significant difference

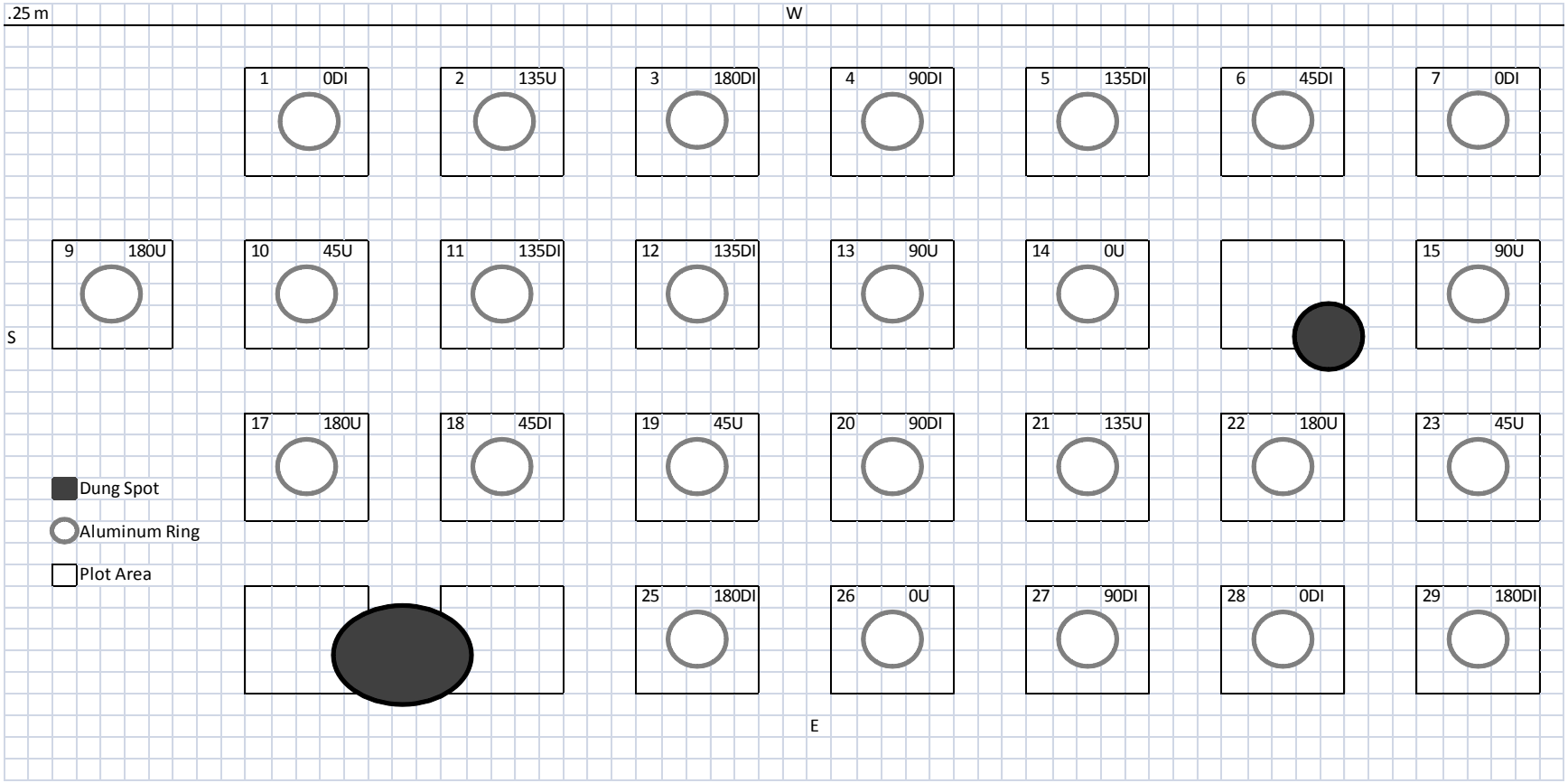


Figure 2.1 2012 plot layout design including plot number in the upper left hand corner of each square plot and treatment in the upper right hand corner. Anchors were installed in the center of each plot as indicated by the silver circles and dung spots were avoided which are indicated by the black outlined and dark filled spots.

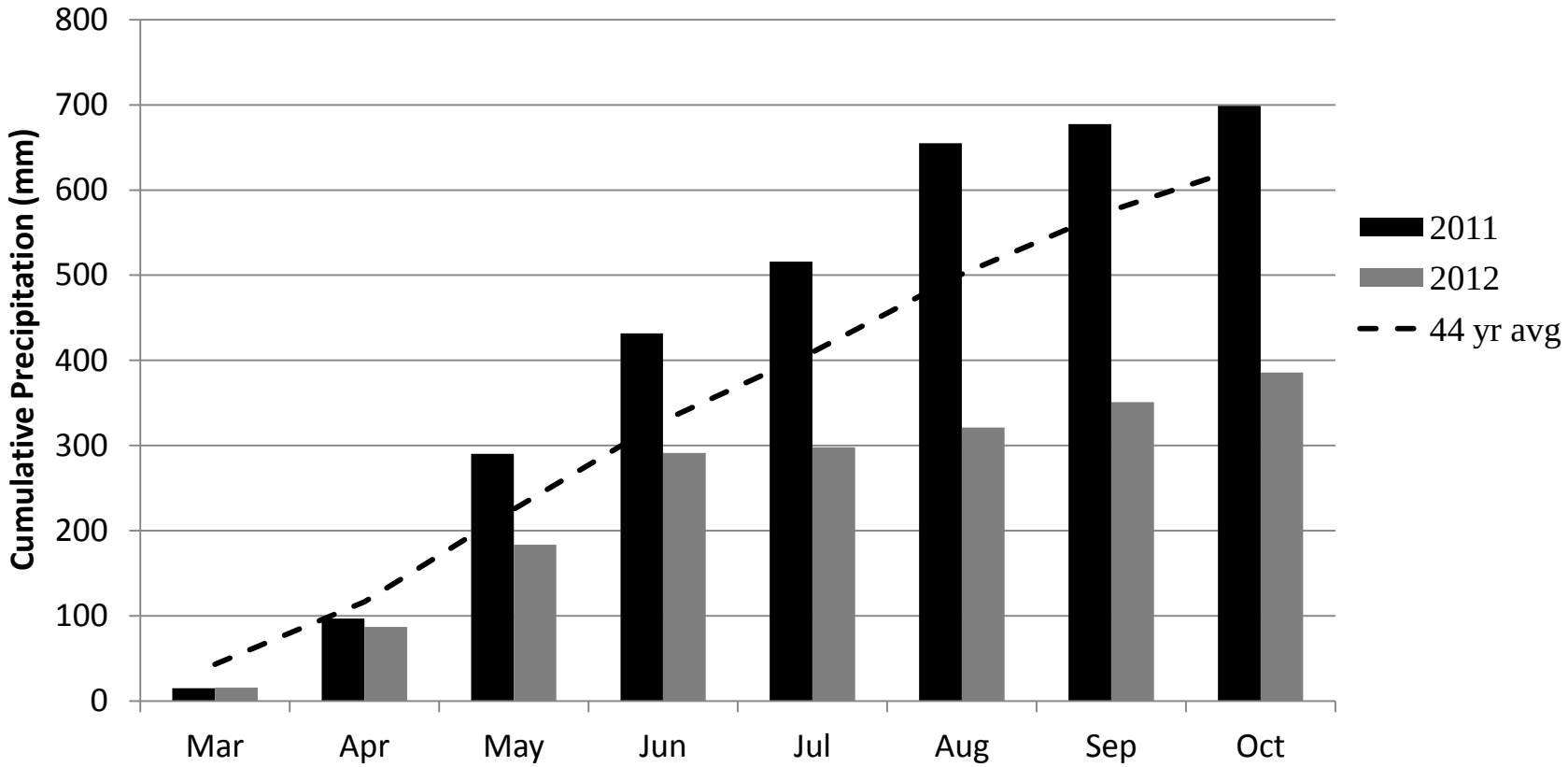


Figure 2.2 Cumulative growing season precipitation by month taken from the High Plains Regional Climate Center station 255362 located less then 2 km from the sampling area. The long-term average has been recorded for this location from 1967-2011. Precipitation in 2011 was above average where as precipitation in 2012 was below average resulting in wide spread extreme drought in the study area.

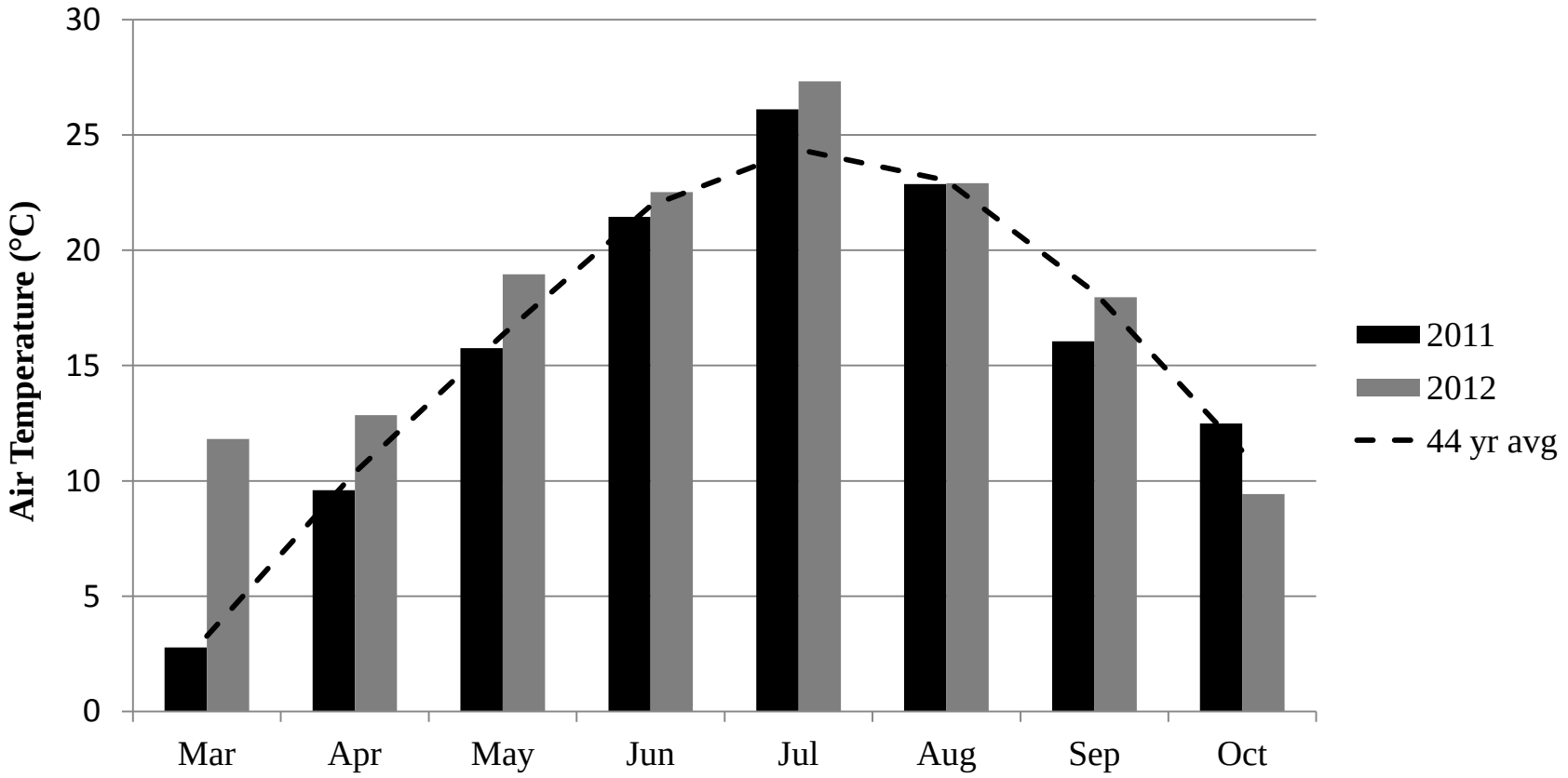


Figure 2.3 Growing season average air temperature by month taken from the High Plains Regional Climate Center station 255362 located less then 2 km from the sampling area. The long-term average has been recorded for this location from 1967-2011. Average air temperatures in 2011 were close to the long term average but air temperatures in 2012 were above average for five of eight months in the growing season which contributed to the "extreme drought" in the area.

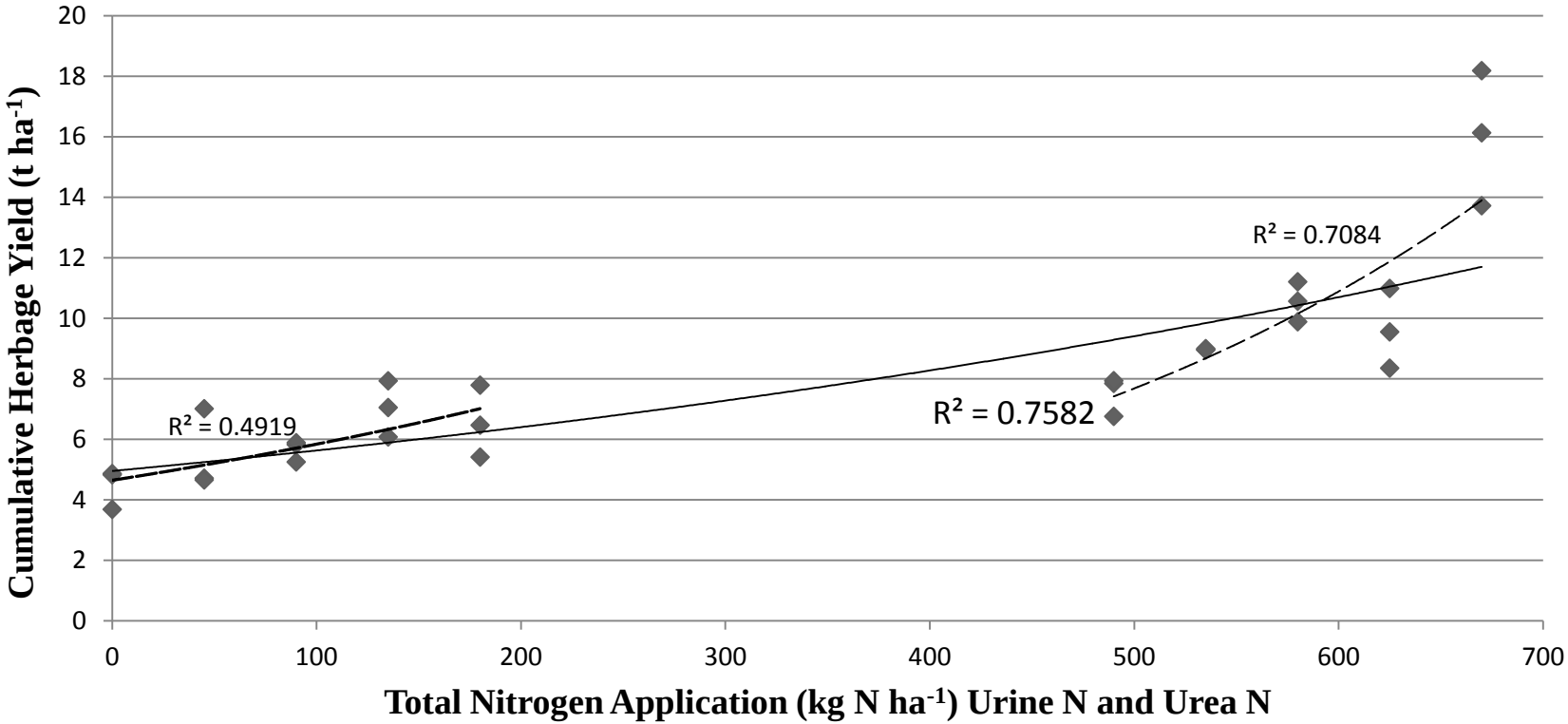


Figure 2.4a Cumulative herbage yield in tons ha^{-1} in 2011 and corresponding total N application from urine N and urea N. The solid trendline shows an exponential increase of cumulative herbage yield as total N inputs increase. Trendlines for the two urine treatments, shown as dashed lines, indicate that as nitrogen inputs increase, cumulative herbage yield increases exponentially.

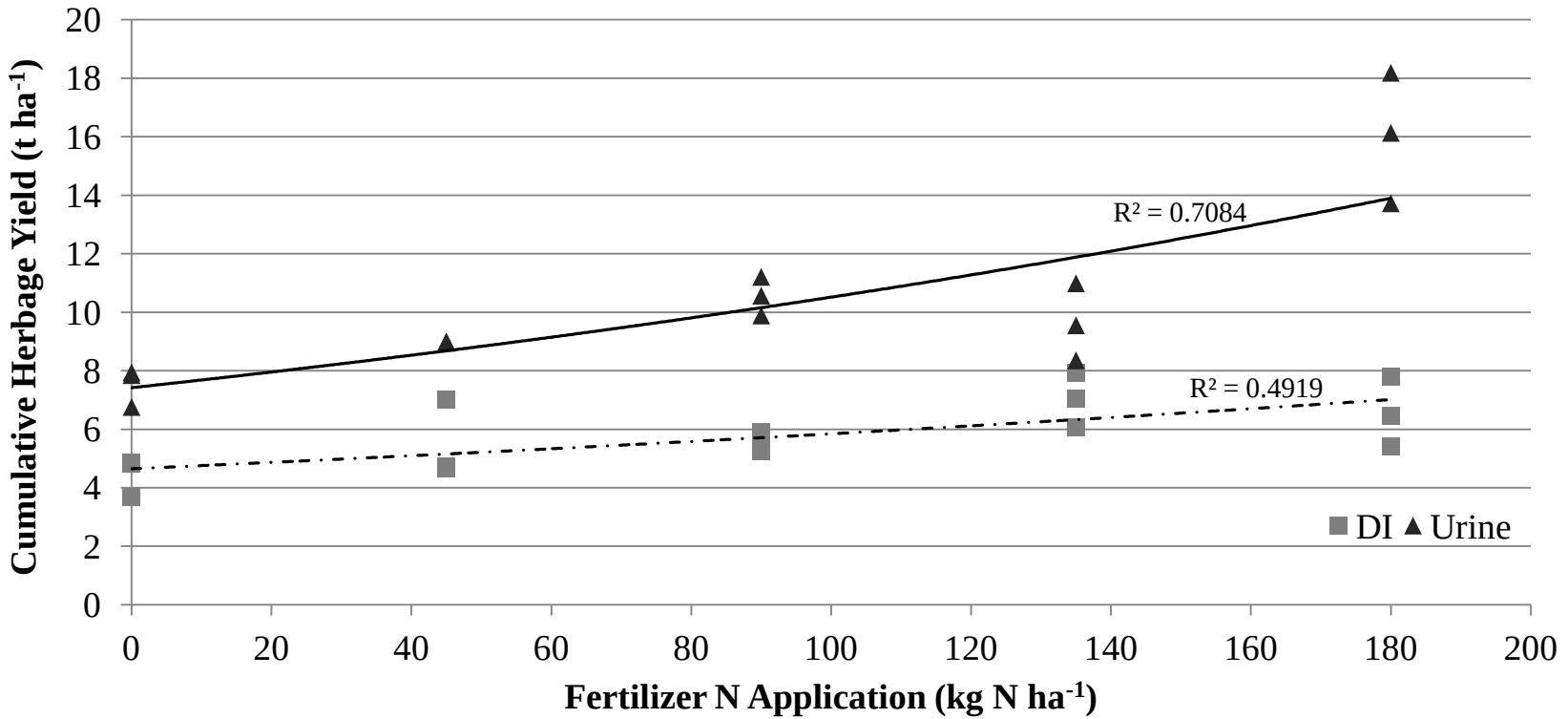


Figure 2.4b Cumulative herbage yield in tons ha^{-1} in 2011 and corresponding fertilizer application rate. Trendlines for each urine treatment show an exponential increase in herbage yield as fertilizer application rate increases.

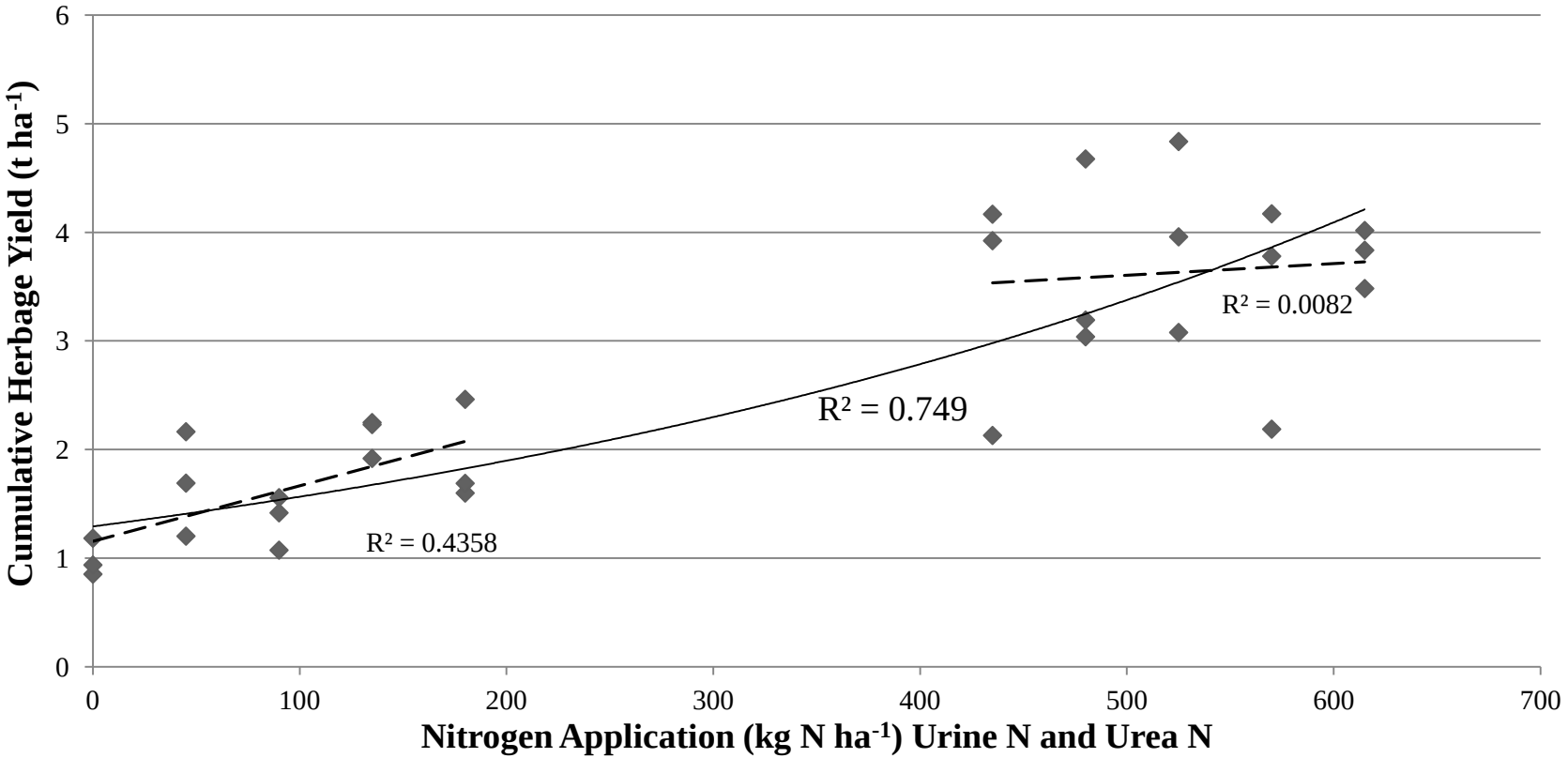


Figure 2.5a Cumulative herbage yield in tons ha^{-1} in 2012 and corresponding total N application from urine N and urea N. The solid trendline shows an exponential increase of cumulative herbage yield as total N inputs increase. Trendlines for each urine treatment show an exponential increase in cumulative herbage yield as fertilizer application rate increases for the distilled water treatment but no significant change in cumulative herbage yield with increased fertilizer rates in the urine treatment. Cumulative herbage yield plateaus in 2012 with additional N application of over 435 kg N ha^{-1}.

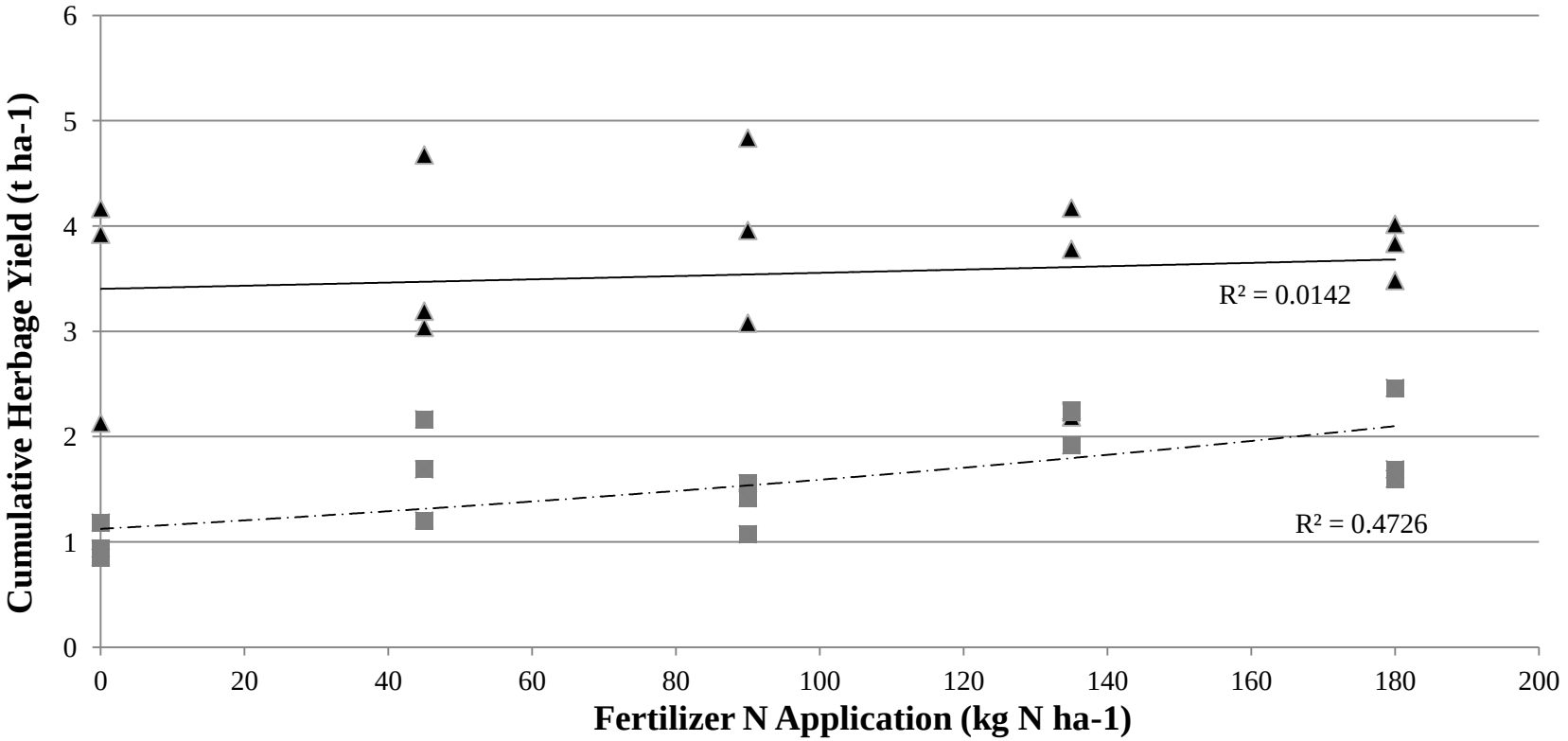


Figure 2.5b Cumulative herbage yield in tons ha^{-1} in 2012 and corresponding fertilizer application rate. Trendlines for each urine treatment show an exponential increase in cumulative herbage yield as fertilizer application rate increases for the distilled water treatment but no significant change in cumulative herbage yield with increased fertilizer rates in the urine treatment.

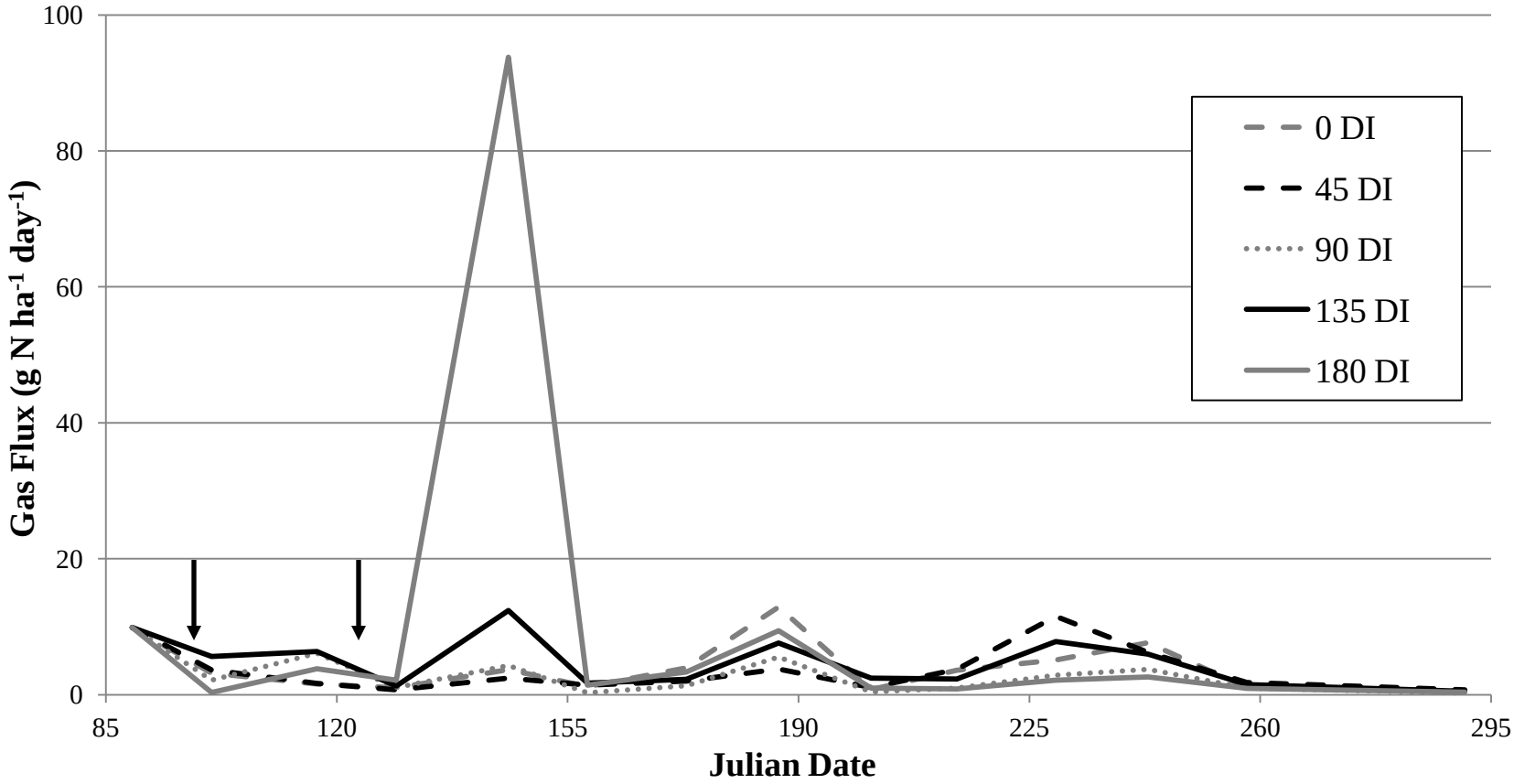


Figure 2.6a Daily flux rates in g N ha^{-1} day^{-1} for each N fertilizer and distilled water treatment in 2011 and the date of sampling. Notice the large peak flux on day 146. Arrows indicate when nitrogen fertilizer was applied on 7 April 2011 (97) and urine application occurred 4 May 2011 (124).

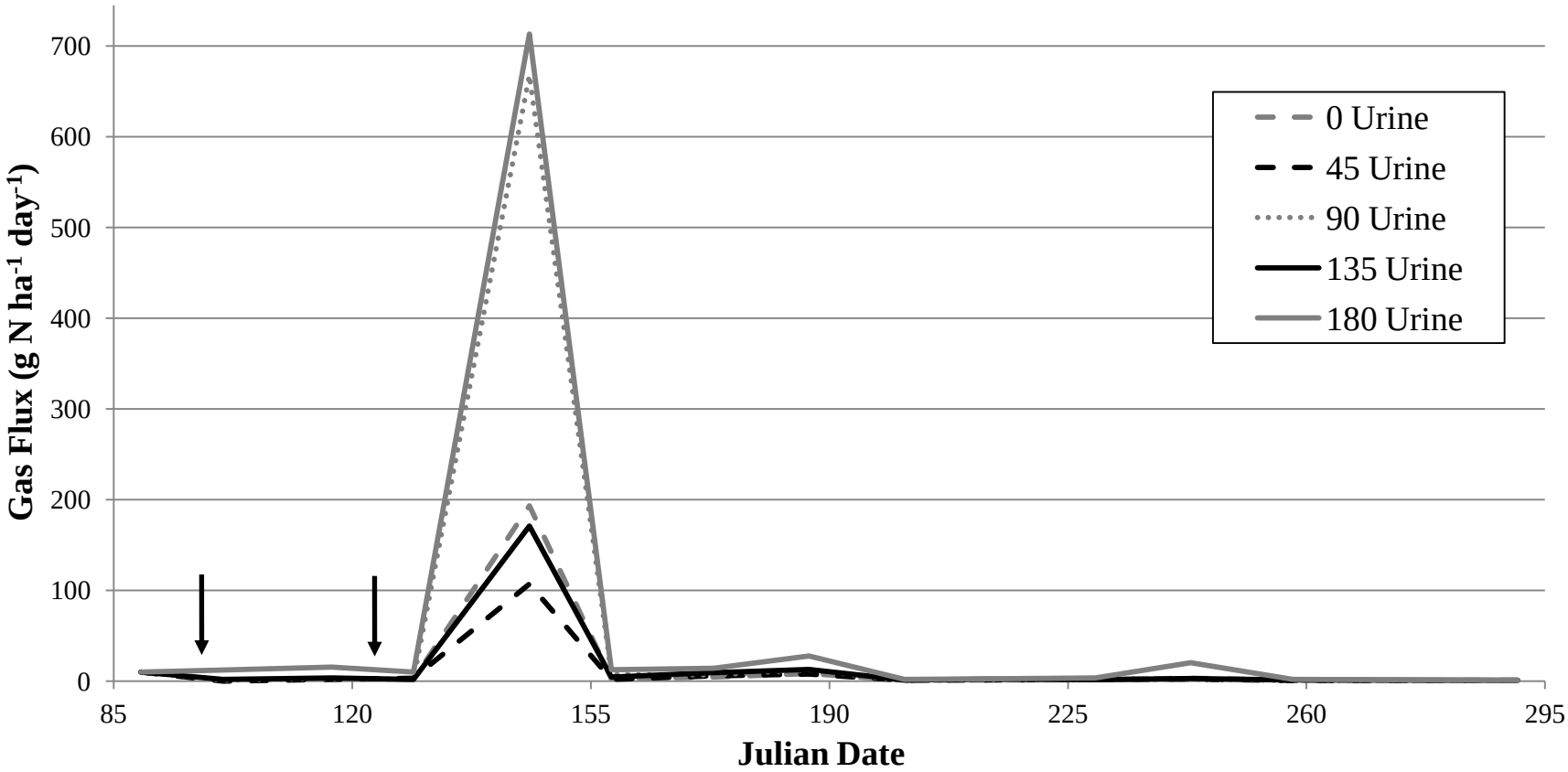


Figure 2.6b Daily flux rates in g N ha^{-1} day^{-1} for each N fertilizer and urine treatment in 2011 and the date of sampling. Notice the large peak flux on day 146. Arrows indicate when nitrogen fertilizer was applied on 7 April 2011 (97) and urine application occurred 4 May 2011 (124).

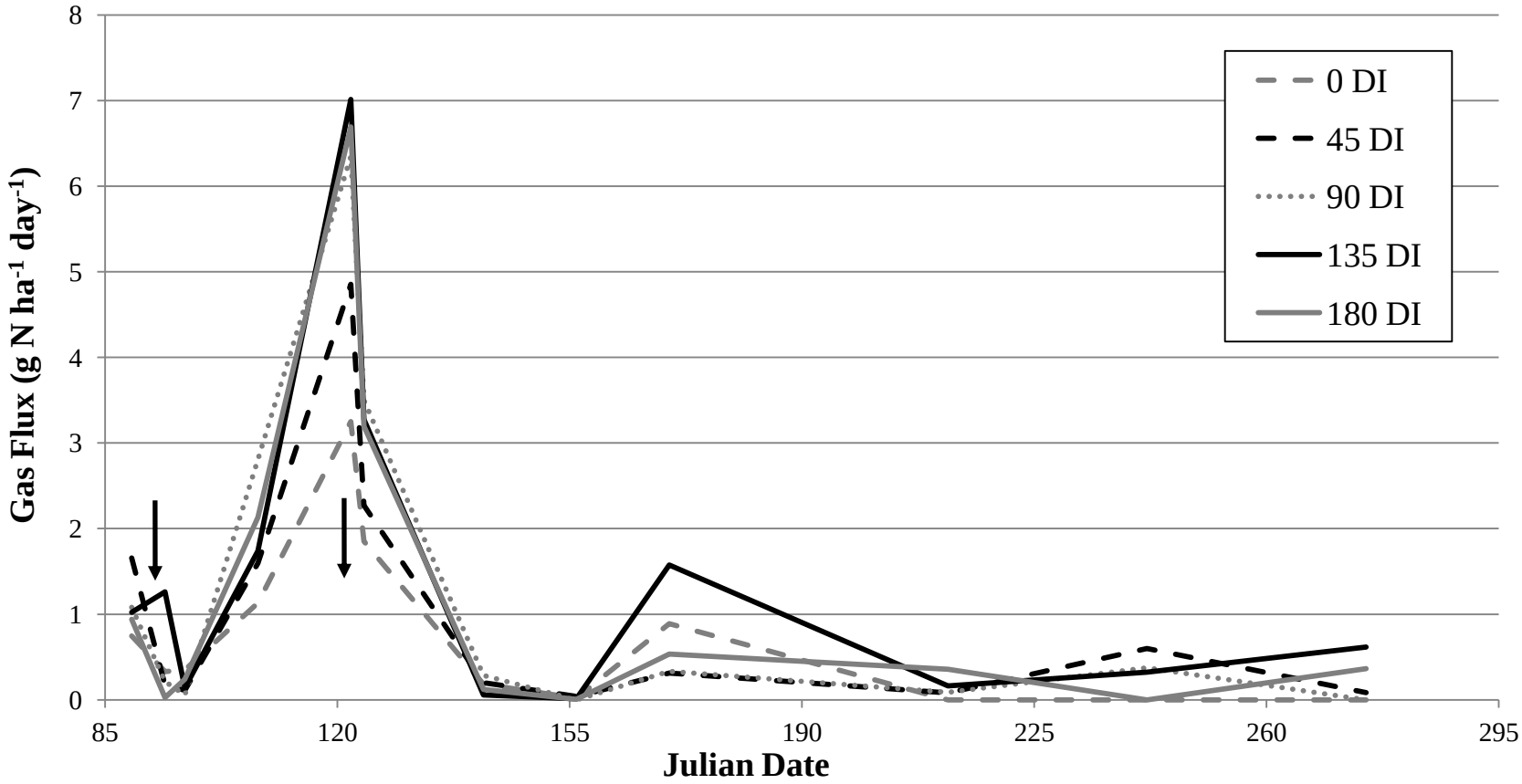


Figure 2.7a Daily flux rates in g N ha^{-1} day^{-1} for each N fertilizer and distilled water treatment in 2012 and the date of sampling. No peak was caught like in 2011 and fluxes were much lower. Arrows indicate when nitrogen fertilizer was applied on 3 April 2012 (94) and urine application occurred and 2 May 2012 (123).

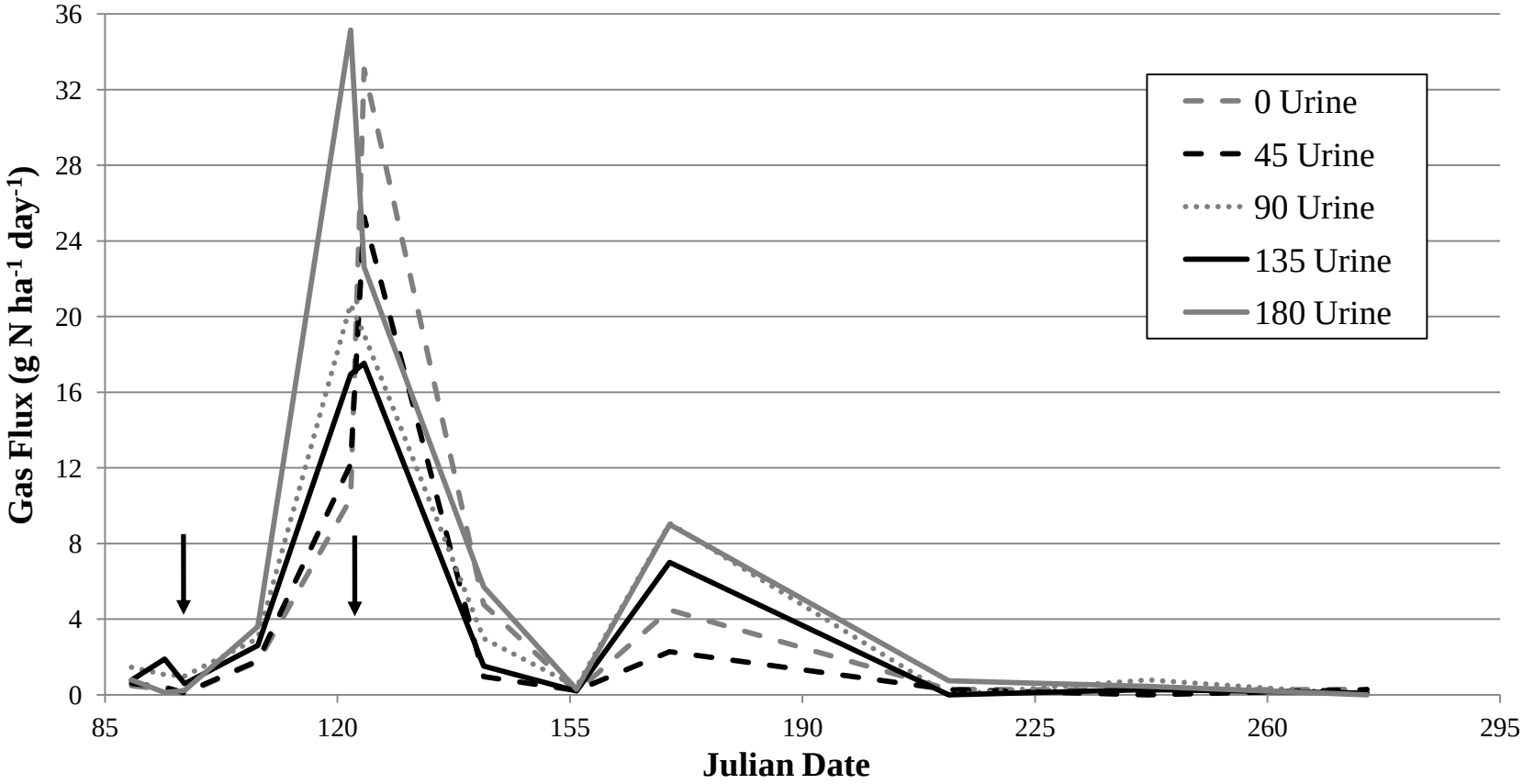


Figure 2.7b Daily flux rates in g N ha^{-1} day^{-1} for each N fertilizer and urine treatment in 2012 and the date of sampling. No peak was caught like in 2011 and fluxes were much lower. Arrows indicate when nitrogen fertilizer was applied on 3 April 2012 (94) and urine application occurred and 2 May 2012 (123).

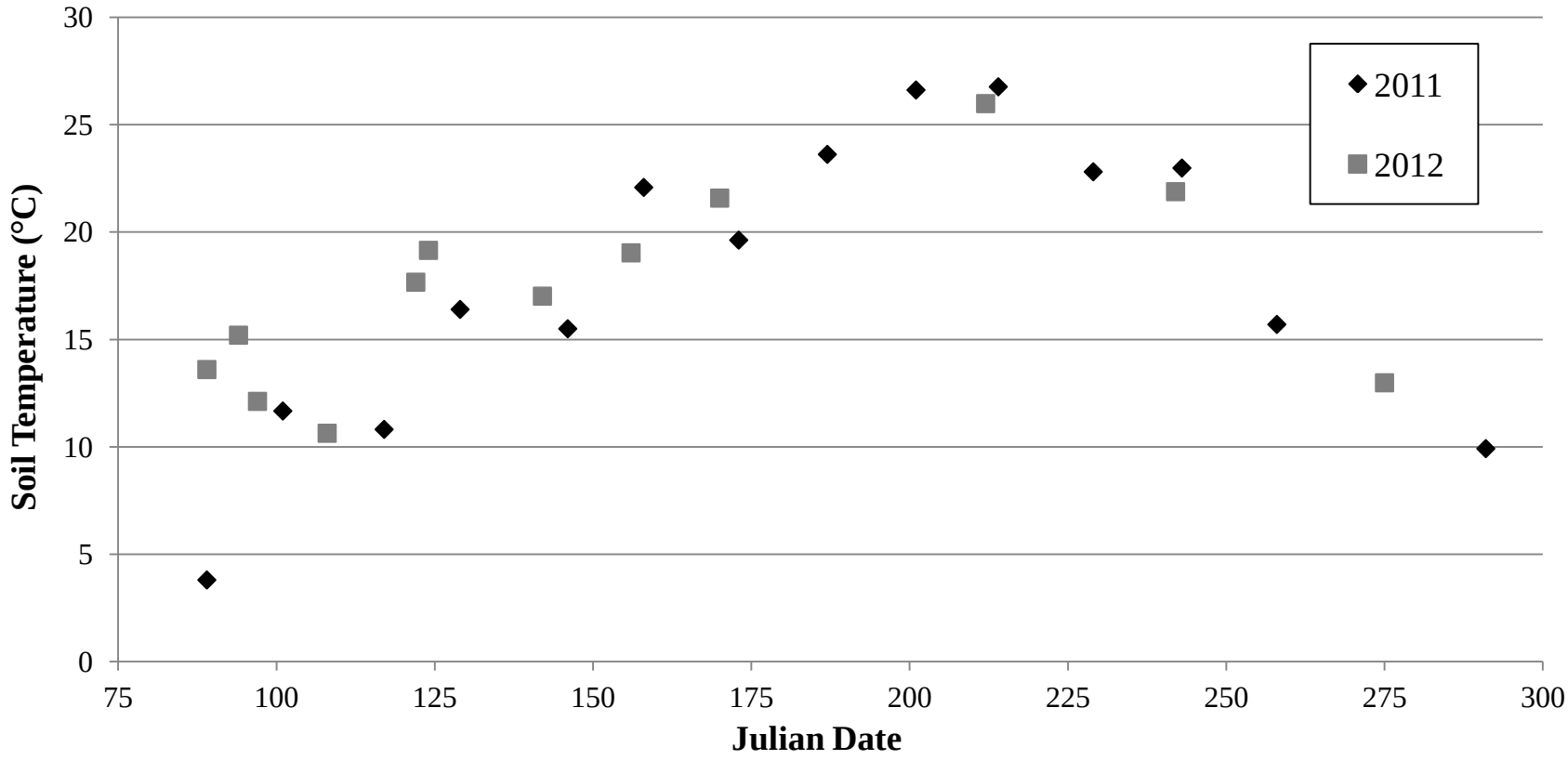


Figure 2.8 The variability of soil temperature over the growing season in 2011 and 2012. Soil temperatures were warmer in 2012 than 2011 in March and April but were very similar in the middle and end of the growing season.

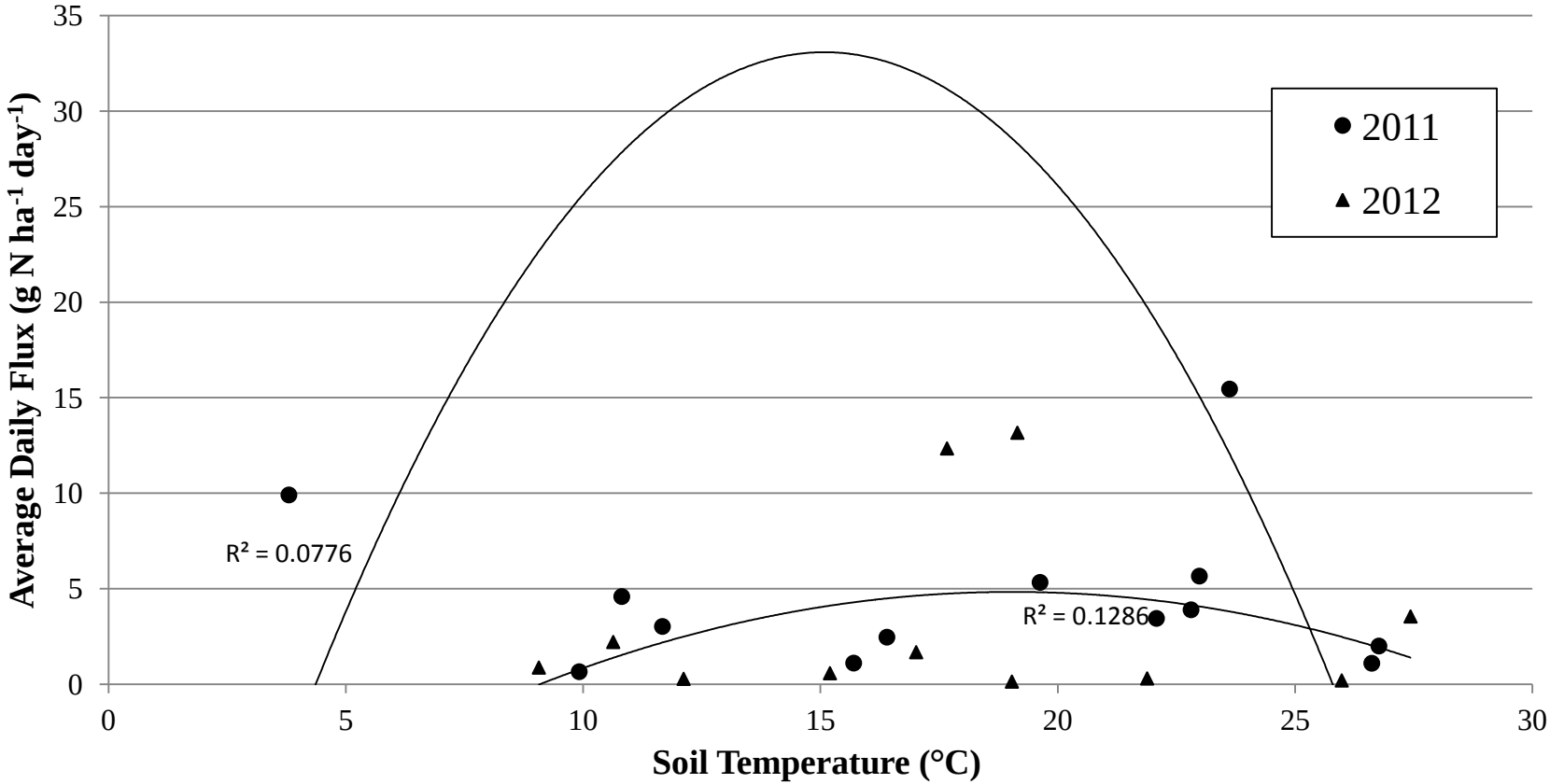


Figure 2.9 There was no reconizable relationship between average daily flux and soil temperature in 2011 or 2012.

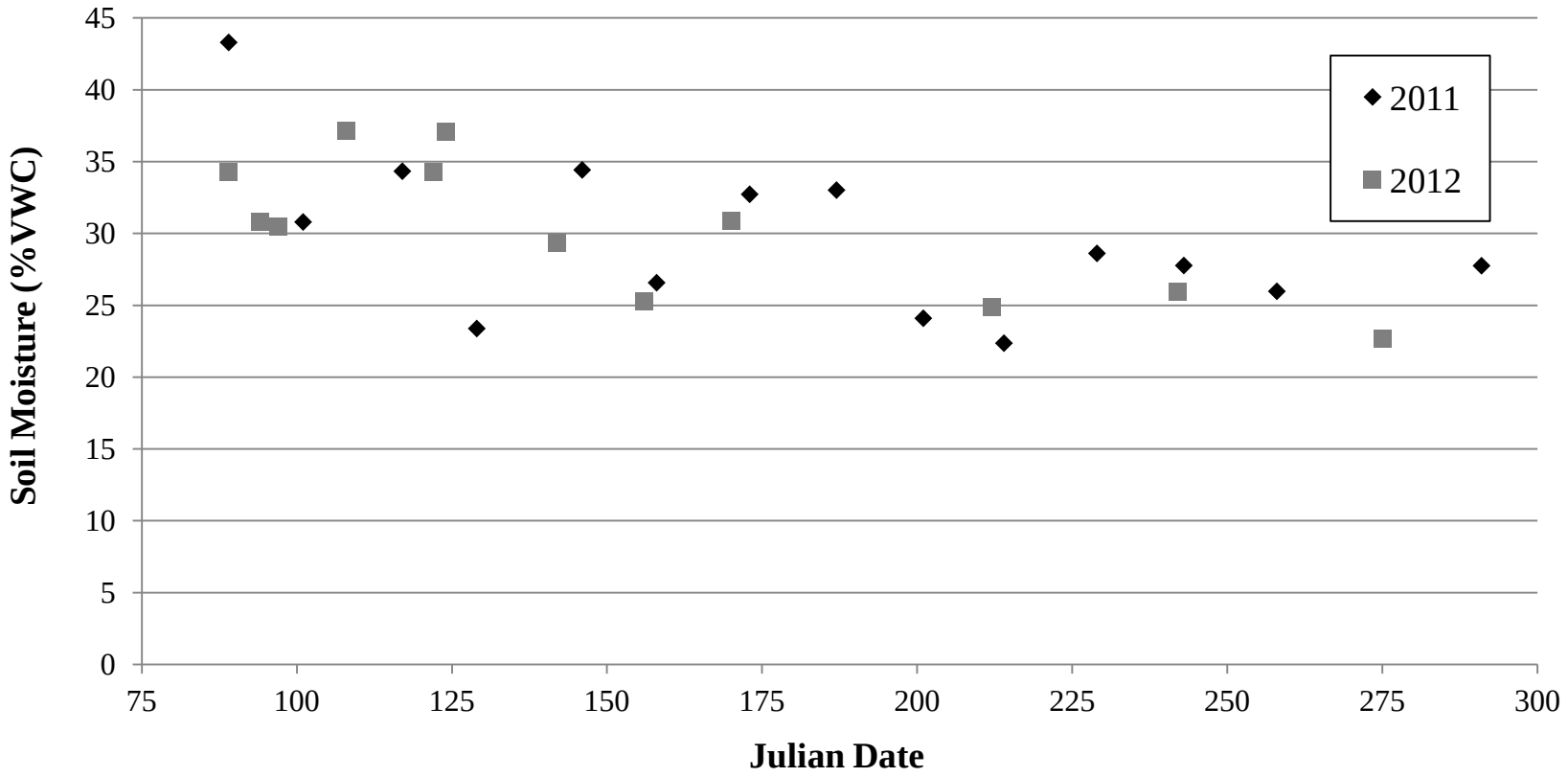


Figure 2.10 The changes in soil moisture in the 2011 and 2012 growing seasons. Soil moistures are highest at the beginning of the season and lowest at the end of the season.

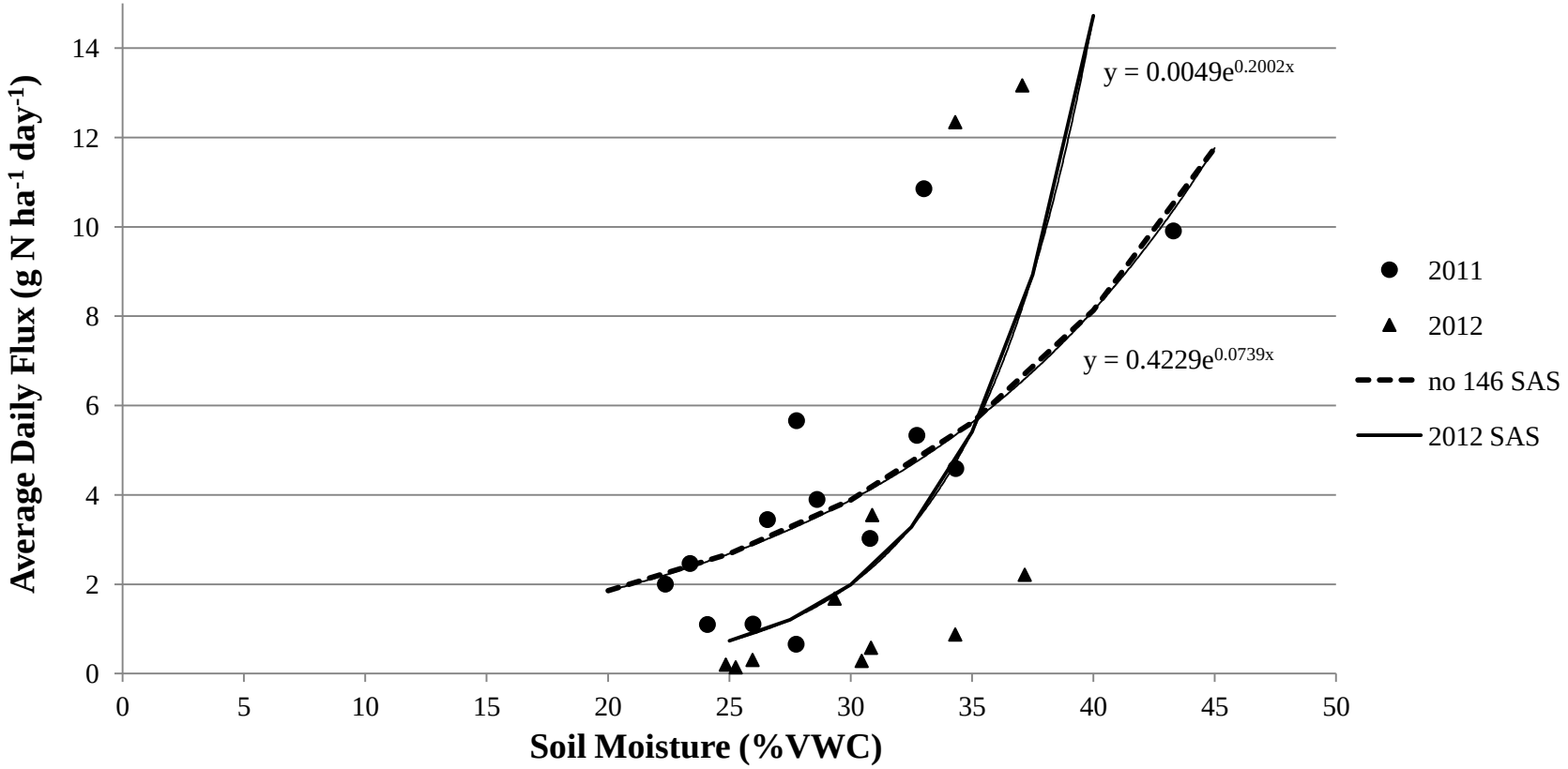


Figure 2.11 There is a significant exponential relationship between soil moisture and average daily flux in 2012 ($p = 0.0125$) and in 2011 when day 146 is removed ($p = 0.0001$).

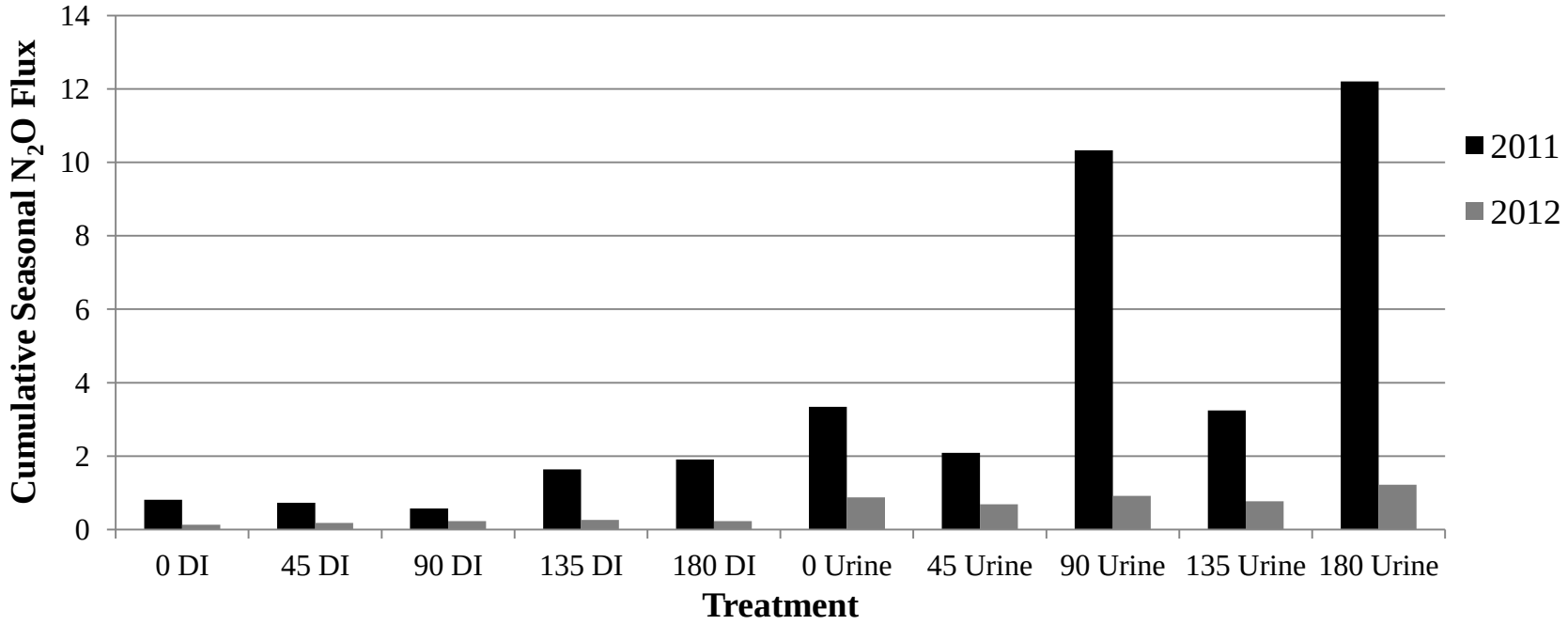


Figure 2.12 Cumulative seasonal N_2O fluxes by treatment for 2011 and 2012. Cumulative fluxes in 2012 were much lower than in 2011. Although the 90 kg N fertilizer with urine and 180 kg N fertilizer with urine treatments showed much higher fluxes than the rest of the treatments, the 135 kg N fertilizer with urine treatment did not show the same pattern.

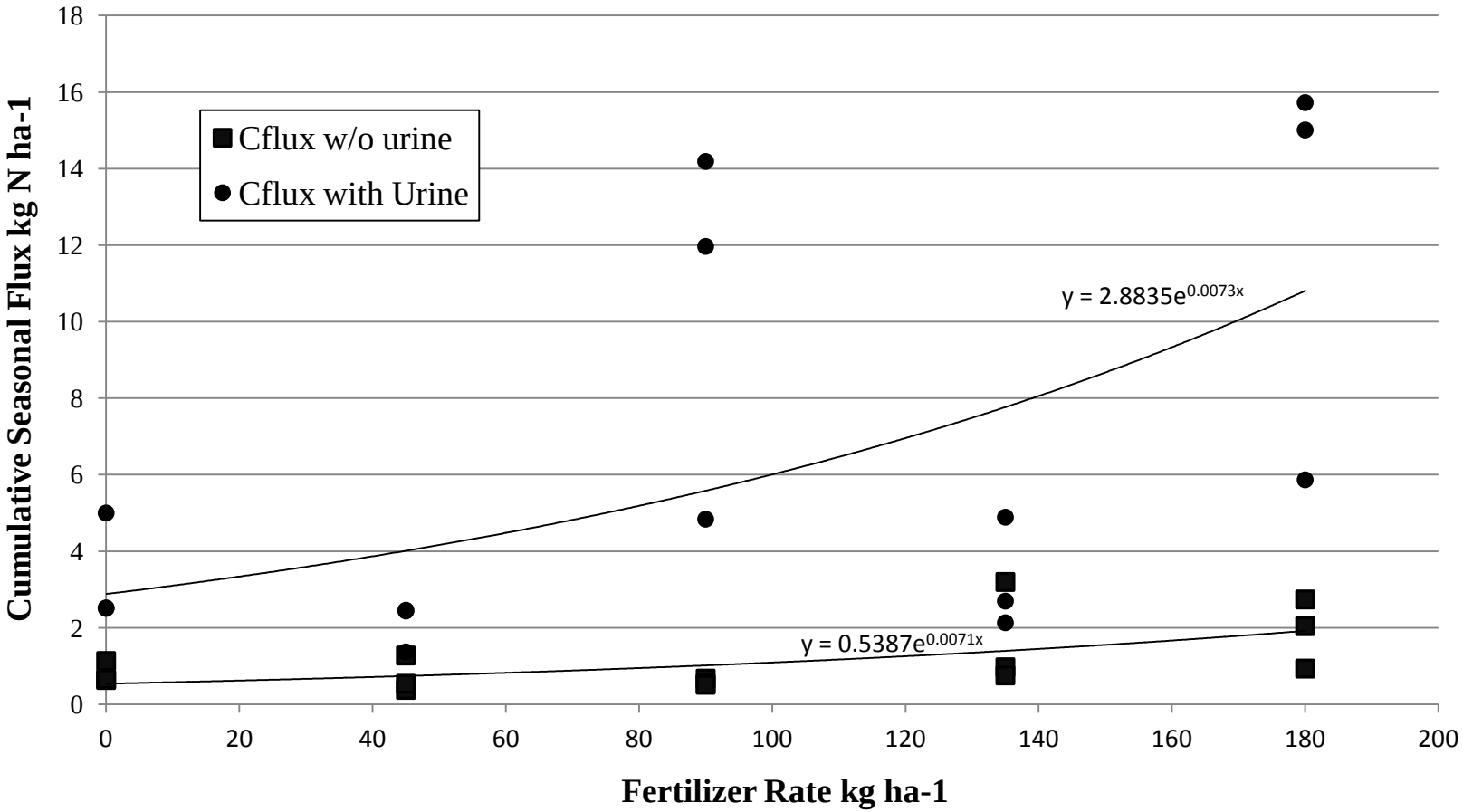


Figure 2.13 Cumulative seasonal fluxes in 2011 were exponentially correlated with fertilizer rate in respect to urine application at $p < 0.05$. With more research, these exponential lines could be used to predict N_2O emissions in smooth bromegrass pasture when environmental variables, fertilizer application, and urine application are known.

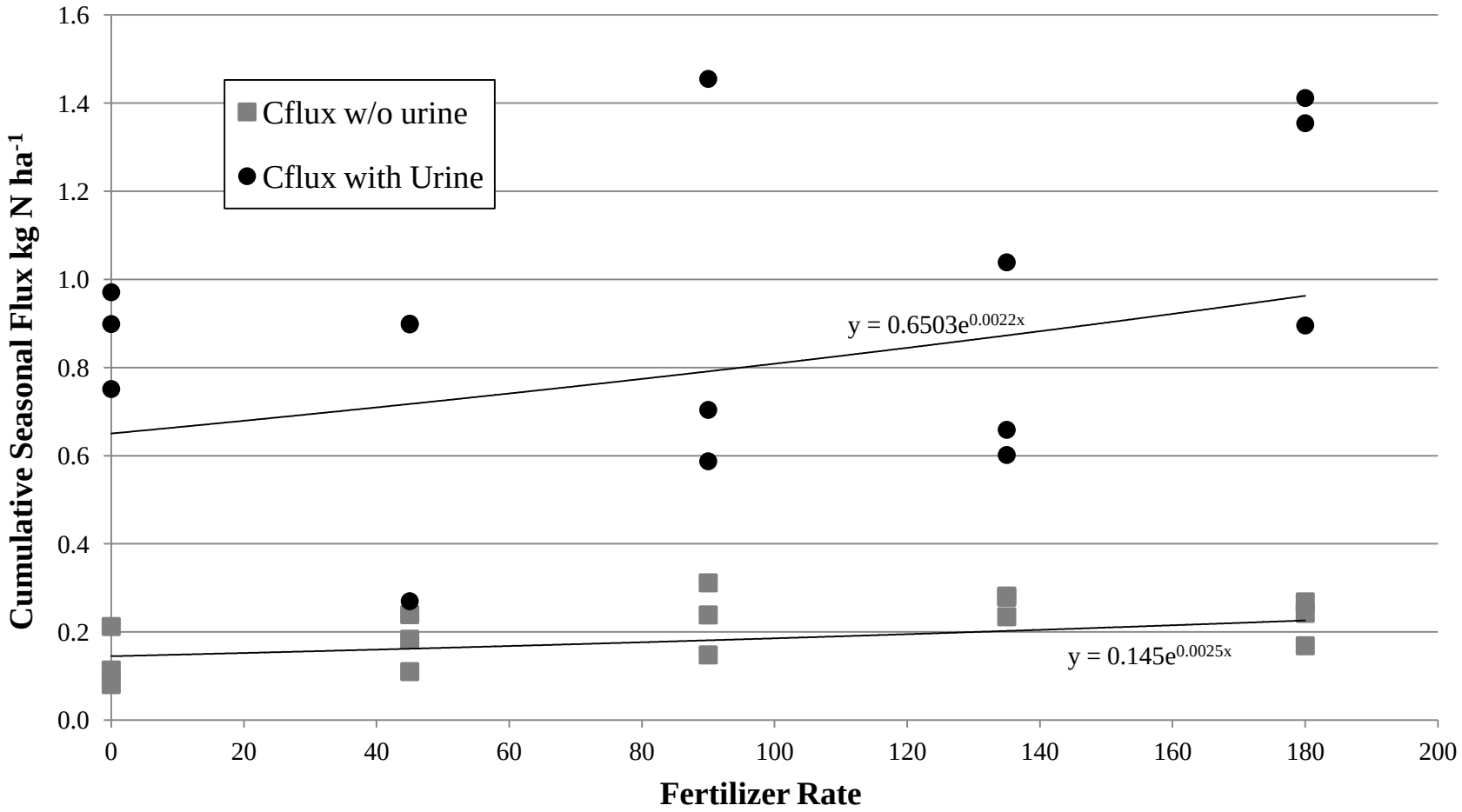


Figure 2.14 Cumulative seasonal fluxes in 2012 were exponentially correlated with fertilizer rate in respect to urine application at $p < 0.05$. With more research, these exponential lines could be used to predict N_2O emissions in smooth bromegrass pasture when environmental variables, fertilizer application, and urine application are known.

Calculating the proportion of a pasture being affected by at least one urination event given stocking rate, urinations per hour, and expected area per urination (Eq. 2.1)

$$t = \frac{\ln(1-x)}{\ln(1-s\lambda\mu)}$$

Where t is the time that the grazing animals spend on the pasture, x is the proportion of the pasture affected by at least one urination event, s is the number of grazing animals, λ is the number of urinations per hour, and μ is the area affected by each urination event. Adapted from Pleasants et al. (2007).