

NITROGEN MANAGEMENT FOR CLIMATE
CHANGE MITIGATION IN THE SOUTHERN GREAT
PLAINS

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2011

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2014

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Date of Degree: DECEMBER, 2014

Title of Study: NITROGEN MANAGEMENT FOR CLIMATE CHANGE
MITIGATION IN THE SOUTHERN GREAT PLAINS

Major Field: Soil Science

Abstract: Nitrous oxide (N_2O) is a potent greenhouse gas (GHG), with a global warming potential 310 times that of carbon dioxide (CO_2). Agricultural soil management in the U.S. is responsible for 69% of N_2O emissions. Fertilizer induced N_2O emissions (the difference between fertilized and unfertilized soil) are estimated to be $1.25\% \pm 1.0\%$ of N applied to agricultural fields. Cellulosic biofuel has been promoted as a method of reducing GHG emissions from the combustion of fossil fuels. However, there is little data available to evaluate N_2O emissions from biofuel feedstock production such as forage sorghum and switchgrass. Therefore a study was conducted in Stillwater, OK to measure N_2O emissions from the potential biofuel feedstocks; forage sorghum, switchgrass, and mixed grasses over 3 years. There are few studies examining the basic effects of N fertilization on N_2O emissions from dry land winter wheat in semi-arid environments. The southern Great Plains of the U.S. has no data evaluating the impact of N application rate on N_2O emission from winter wheat. Thus, this winter wheat production area, representing 20.9 million acres is not represented within global N_2O emission estimates used by the IPCC. Therefore, a study was established in a long term continuous winter wheat fertility experiment in Stillwater, OK to determine the effects of N rate on N_2O emissions from dry land winter wheat in the southern Great Plains of the U.S. in order to fill this knowledge gap. Legume cover crops have been used to fix N from the atmosphere and have been suggested as a method to reduce N fertilizer inputs. Little research has been focused on evaluating the use of cover crop mixtures. Therefore a study to evaluate the impacts of using leguminous cover crop mixtures on N cycling, soil moisture, and cash crop performance in continuous no till winter wheat production was established in 2013. Emissions of N_2O are highly variable and depend greatly on climatic conditions and are influenced by N fertilization. Cover crops did not impact wheat yields, and cover crop mixtures with grass species as a component reduced soil NO_3 levels more than legume only mixtures.

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CHAPTER I

LITERATURE REVIEW

Nitrogen

Nitrogen, phosphorus and potassium are the three nutrients most commonly deficient, of these macronutrients, N is by far the most limiting nutrient as it is relatively mobile in soils and therefore subject to losses. Nitrogen is a critical component of amino acids which are the foundation upon which proteins are built. These proteins are in the form of various enzymes which drive metabolic reactions in plants (Oklahoma Soil Fertility Handbook, 2006). In order to sustain high production of crops used for food, fiber and fuel, supplemental N must be added to the system. In 1950, the world produced 631 million tons of grain. Fifty years later, world grain production had increased to 1,840 million tons in 2000 (Mosier et al., 2004). The world population is expected to increase nearly 50% between 2000 and 2050 (United Nations, 2004). This increase in population means even more mouths to feed on increasingly limited resources, such as land and water. In the past, this magnitude of increase required major innovations and adaptations to agriculture, i.e. the “Green Revolution,” in order to meet demand for food production; further innovations and adaptations are required now (Pimentel, et al., 1976). The global land area suitable for crop production is a mere 11 percent. In the US, the best agricultural land is already in use; yet as urbanization spreads, more and more arable land

is lost to highways and urban developments (Pimentel, et al., 1976). With an expanding population to feed on increasingly limited land, the efficiency of every single acre producing food or feed must increase.

Global Nitrogen Cycle

Since the development of the Haber-Bosch method of fixing N_2 from the atmosphere, mankind has been able to greatly increase the productivity of the land by applying inorganic N. However, this ability to artificially fix N has altered the natural N cycle. Globally, approximately 130 terragrams (Tg) of N is fixed biologically each year with terrestrial fixation accounting for 100 Tg N, ~20 Tg N from marine ecosystems and ~10 Tg N fixed by lightning (Vitousek, 1994). Nitrogen fixation from human activity has added approximately 80 Tg annually to the global N cycle through industrial N fixation (Haber-Bosch method), 25 Tg of N (as NO_x) is released by the combustion of fossil fuels and approximately 30 Tg of N is added from leguminous crops (above background N fixation on lands). The amount of N fixed by human processes now outpaces the amount of N fixed by nature. Not only have human processes contributed to an increase in N fixation, the cycling of N has also been sped up by the draining of wetlands, land use change and burning of biomass. Slow decomposition rates in wetland systems allows for the wetland to be a sink for nutrients, when a wetland is drained the N stored becomes subject to mineralization and thus is potentially released into the environment as NO_3 in ground water and NH_3 , NO_x , and N_2O in the atmosphere (Schlesinger, 1997). Land use change alters the rate of emissions from land. When a forest or grassland is converted to agricultural production, the benefits of those ecosystems are lost. Forests and grasslands are able to act as carbon (C) sinks, storing C in the soil and in the biomass they produce. Conversion of these ecosystems to agricultural

use not only removes the ability to store C in the soil but N is then added to boost yields of the crops grown on the land which increases the amount of N lost as N_2O gas and/or N lost to leaching (Searchinger, et al., 2008). Burning of biomass on nutrient rich soil can stimulate N_2O and NO production (Anderson, et al., 1988). Runoff and erosion are often increased following fire which provides another pathway for N to leave the system.

The effects of anthropogenic N production outpacing background fixation are varied and far reaching such as: damage to surface water bodies, ground water contamination, damage to the ozone, loss of plant diversity, etc. (Galloway, et al., 2008). Eutrophication of surface waters is a result of an increase in plant nutrients (N and P) that results in a flush of growth of algae in the water body. Decomposition of these algae after death depletes the dissolved oxygen (O_2) leading to the death of aerobic organisms. Eutrophication not only impacts the organisms living in the body of water but will also impact other uses of the body of water such as agricultural, industrial, municipal or recreational use. Excessive nitrates (NO_3) from N fertilizer leaching through the soil profile into ground water used for drinking has been found to cause serious health conditions such as methemoglobinemia or “blue baby” syndrome in infants under 6 months old (Comly, 1987). By increasing the amount of N available in ecosystems, the number of species found (species density) declines (Vitousek, et al., 1997). In native prairies the addition of N fertilizer has been found to increase the annual net primary productivity (ANPP) yet decrease the species density or number of species found (Gough, et al., 2000).

Globally, it is estimated that 14.4% of all N fertilizer applied is lost to the atmosphere as ammonia (NH_3) (Mosier, et al., 2004). Ammonia (NH_3) volatilization from urea application is of particular concern in No-Till systems, soil with high pH, and when temperatures are

warm. Ammonia is produced when an amine group (NH_2) goes through the ammonification process to become NH_3 . At soil pH below 7.0, the NH_3 is either converted into solid ammonium (NH_4) and is then in the soil system and can undergo nitrification to become NO_3 or is fixed to exchange sites on the soil minerals. When the soil pH or microsite pH is above 7.0, the NH_3 is not converted to NH_4 and is more likely to be volatilized into the atmosphere. The rate of volatilization is greater with increased N rates and temperatures (Overrein and Moe, 1967). Not only is the loss of N as NH_3 gas an inefficiency in the fertilization system, but it also can contribute to regional smog (Mosier, et al., 2004).

Nitrogen Use Efficiency

In addition to the demand for increased efficiency of food production is the demand for producers to become better stewards of the land in order to minimize the harmful environmental effects of increasing food production. The best way to achieve this balance of environmental stewardship and production increase is to improve nitrogen use efficiency (NUE). Options for improving NUE include, introducing crop rotations, using controlled release fertilizers (CRF) or nitrification inhibitors (NI), banding or subsurface placement of N fertilizer, using $\text{NH}_4\text{-N}$ as the N source for fertilization, using in-season N applications or foliar applied N, and using precision agriculture practices.

Diverse crop rotations (three or more species) allow for improved nutrient cycling as crops vary in nutrient demand, retention, and release of nutrients (Blanco-Canqui, et al., 2008).

This variation in nutrient requirements and cycling can help prevent the loss of NO_3 to leaching (Delgado, et al., 2001). In a monocropped system, N is made available to the crop for use during the growing season but if that N is not utilized by the crop then it remains in

the soil profile during the fallow season until enough rain comes along to leach it below the rooting zone where there is no chance to recover it. Ideally, any “leftover” N would remain in the rooting zone during fallow waiting to be used by the next crop. Since “ideal” is rarely reality, crop rotations allow for a crop to be in the field to “catch” N to prevent leaching or even fix N in the case of legumes used in rotations. By keeping the N in the soil/crop system, the NUE of the system is increased.

An experiment in northeastern Colorado by Shoji, et al. (2001) showed the use of banded CRF and NI on irrigated barley has the potential to significantly increase NUE compared to banded urea. For a no-till system in Central Oklahoma, Rao and Dao (1996) found that banding urea in seed rows or between rows increased yield by 32% and 15%, respectively, compared to broadcasting urea. Grain N content was also increased by 33% for the treatments with N banded in the seed rows and 25% for the treatments with N banded between rows compared to broadcast. This increase in yield and grain N content would result in an improved NUE compared to the broadcast application method. By placing the N fertilizer below the soil surface, the opportunities for loss from volatilization or immobilization are decreased.

The use of $\text{NH}_4\text{-N}$ as the N source has been proposed as a method of improving NUE since NH_4 is immobile in the soil and therefore not susceptible to leaching. However, the conversion of NH_4 to NO_3 is often more rapid than the plant can take it up; meaning the resistance to leaching is only temporary when NH_4 is used as the N source. Another cause for concern for many producers is that when NH_4 undergoes nitrification 2 moles of H^+ are produced. Over time, the use of NH_4 as the N source can lead to acidification of the soil,

increasing production costs for the producer who now must apply lime to reverse the effects of the acidification (Alva, et al., 2006).

Supplying a crop with N when N is in demand by the plant can increase efficient utilization of the fertilizer (Alva, et al., 2006). However, applying at precisely the correct time is not always practical. Producers can still benefit from split applications of N, especially for winter wheat. Applying a small amount of N fertilizer as a “starter” fertilizer or up to half of the full N rate in the fall, then applying the rest of the N in the spring allows the N to be available when it will be in higher demand by the crop. By implementing split applications of N to winter wheat in the Pacific Northwest, Mahler, et al. (1994) recorded NUE of 58-61% compared to NUE of 52-55% for fall only applications and NUE of 51-53% for spring only applications. Differences in NUE and grain yield between N fertilizer sources and placement were not significant, indicating that timing of N fertilizer application for that region plays a larger role in NUE. In Oklahoma, by applying foliar N (34 kg N ha^{-1} UAN) to dryland winter wheat at either pre- or post-flowering was shown to increase grain N content over the check plots showing the potential to increase NUE by, again, supplying the crop with N when it is in high demand and soil conditions cannot be relied upon to supply N to the plant (Woolfolk, et al., 2002).

Precision nutrient management is a rapidly expanding sector in agriculture as producers seek to only apply exactly what is needed, exactly where it is needed. One simple method that requires no special equipment or calculations is the use of N-rich strips or N reference strips which provide producers with a very quick visual assessment of the N status of a crop. Other methods include using the normalized difference vegetation index (NDVI) to monitor N status or variable rate applications of N based on grid soil sampling or management zones

(Alva, et al., 2006). While these methods have been shown to improve N management, the majority require specialized equipment and/or large investments of time and money, which can deter many producers from adopting the practice or method.

Nitrogen management and NUE are complex issues to tackle and the variability from one region to the next or even from one field to the next can be quite high; there is no simple answer to the question of how to improve NUE and maintain yields. However, by combining the practices discussed in this section and adapting them to fit the climatic and economic conditions for a producer, improvements in N management and NUE will be seen without decreasing yields or damaging ecosystems.

Nitrous Oxide

The increase in anthropogenic N in the biosphere has led to an increase in atmospheric nitrous oxide (N_2O), of 12% – 23% since industrialization (Leuenberger, 1992). Although the atmospheric concentration is relatively low (0.32 ppm), N_2O is of particular concern as the global warming potential of this gas is 310 times that of CO_2 (USEPA, 2012). In addition, N_2O has become the primary ozone depleting substance emitted by anthropogenic means (Ravishankara, et al., 2009). Emissions of N_2O are the result of natural processes occurring in the soil. Primarily, N_2O is produced during the microbial process of denitrification in which nitrate (NO_3) is converted to N_2 gas. When NO_3 is not completely converted to the benign N_2 gas, the resulting byproduct is N_2O . Denitrification occurs under conditions of limited oxygen availability in the soil environment. To a lesser extent, N_2O can also be produced during nitrification, which again is a microbial process whereby ammonium (NH_4) is converted to NO_3 (Bremner and Blackmer, 1978). This reaction can occur anytime that NH_4 concentrations, soil moisture and temperature are adequate. Many factors influence

the emission of N₂O such as, soil moisture, temperature, microbial activity, aeration, and organic matter content.

When the concentration of inorganic nitrogen (NO₃ and NH₄) is increased through applying commercial fertilizers or mineralization of organic N sources (manure or cover cropped legumes), N₂O emissions are increased above ambient levels. Agricultural soil management in the U.S. is responsible for 69% of the N₂O emissions for the country, this represents 3% of all GHG's emitted in the country (USEPA, 2012). Fertilizer induced N₂O emissions (the difference between fertilized and unfertilized plots) are estimated to be 1.25% ± 1.0% of N applied to agricultural fields. Indirect additions of atmospheric N₂O as a result of leaching, runoff, NO_x and NH₃ volatilization are estimated to be approximately 0.75% of N applied (Mosier, et al., 1996).

No-till soil management has been touted as a solution to global climate change due to soil's ability to be utilized as a C sink, thereby offsetting CO₂ emissions from fossil fuels and decreasing the amount of soil C that is oxidized to CO₂ through conventional tillage.

However, Six et al. (2004) found that N₂O emissions increase with the adoption of no-till management over the first 20 years compared to conventional tillage with a moldboard plow. In the first 10 years following conversion, the emissions of N₂O were elevated regardless of climate. In the dry climate, which was represented largely by data from the North American Great Plains, emissions were similar between the conventionally tilled fields and the no-till fields. The explanation for the increased emissions in the first decade of conversion is that the increase in water holding capacity stimulates N₂O emissions during that time period.

Mosier and Hutchinson (1981) found that N₂O emissions from a furrow-irrigated corn (*Zea mays* L.) field in Colorado were approximately 1.3% of the 200 kg N ha⁻¹ applied or 2.5 kg N ha⁻¹ for the period of time between mid-May and mid-September. Thirty percent of the N₂O emitted came during the 2 weeks following fertilization as the NH₃ from the fertilizer was undergoing nitrification. The first irrigation event for the field accounted for 59% of the N₂O emitted when low soil oxygen levels provided a favorable environment for denitrification. The remainder of N₂O emissions occurred rapidly following precipitation or irrigation events greater than 0.7 cm although the emissions from these events were much smaller. Soil water content was strongly correlated to N₂O emissions yet NO₃ in the soil was not since high NO₃ concentrations in soil alone do not cause denitrification to occur.

In the Northern Great Plains region of the U.S. it was found that 4 different fertilized cropping systems all exhibited similar trends in N₂O emissions. The following crop systems were evaluated for N₂O emissions over a 2 year period: conventional tillage (CT) winter wheat (*Triticum Aestivum* L.) -fallow, No-Till (NT) winter wheat-fallow, NT winter wheat-spring wheat, NT winter wheat-spring pea (*Pisum sativum*), and alfalfa (*Medicago sativa* L.) -perennial grass (control). All systems except the alfalfa system were fertilized with a low (0 kg N ha⁻¹), a moderate (100 kg N ha⁻¹), and a high (200 kg N ha⁻¹) N-rate. Following fertilization all systems except the alfalfa-grass had N₂O emissions above the background N₂O levels for approximately 10 weeks in the spring and even longer for a fall application. Elevated N₂O flux was also measured during freeze-thaw cycles in the winter and spring. The post-fertilization periods and the freeze-thaw cycles accounted for the majority of the emissions during the 2 year study. Fertilizer induced emissions made up the largest fraction of emissions with significant differences between the moderate and high rates for the CT and

NT wheat-fallow and significant differences between all three rates for NT wheat-wheat and NT wheat-pea systems (Dusenbury, et al., 2008).

Improving N Management

The nitrogen use efficiency (NUE) for the world is at 33% (Raun and Johnson, 1999).

Several methods for improving NUE are reviewed by Raun and Johnson (1999) such as: introducing crop rotations, banding or subsurface placement of N fertilizer, use of $\text{NH}_4\text{-N}$ as the N source for fertilization, using in-season N applications or foliar applied N, and using precision agriculture practices. Raun and Johnson (1999) concluded that there is not a standalone solution that will sufficiently improve NUE, but rather some combination of the various practices that are appropriate to the producer's climate and production system. A by-product of improved NUE is the decreased risk of environmental harm as a result of N applications to agricultural land.

The environmental and health concerns of over applying N, paired with the damage to yields by under applying N creates a fine line that producers must walk in order to get the maximum yield with minimal harm to the environment. Emission of N_2O is not a local, regional or even statewide issue. Nitrous oxide is a global contaminant; this makes it a much more difficult pollutant to manage. Simply decreasing N rates is not a sustainable option for the world. As food demand soars, any effort to reduce emissions cannot reduce yields. The only real alternative available to reduce N_2O emissions globally is to improve NUE of cropping systems.

Cover Crops

Cover crops have long been utilized for erosion control and prevention. The use of cover crops provides ecosystems services beyond simple erosion protection. These services include improving water quality, suppressing weeds, preventing leaching of mobile nutrients, increasing soil organic matter, increasing crop yields, fixing N and recycling nutrients within the soil (Winger, et al., 2012). No-till systems stand to reap the greatest benefits from cover cropping. The residue left behind after a cover crop helps to buffer the soil from large changes in temperature and moisture content, providing a more favorable seed bed at planting as well as reducing water lost to evaporation. By planting cover crops, the water that would be lost to evaporation during the fallow period can be put to use to produce additional residue and nitrogen when a legume cover crop is used, and to improve soil structure by maintaining an actively growing root system during the fallow period.

Suppression of weeds is an important benefit to using cover crops. According to the National Agricultural Statistics Service in 2007, approximately 6 million acres of cropland in Oklahoma was treated with herbicide. With growing concern over herbicide resistant weeds, implementing cover crops is the best practice to fight resistant weeds and the increasing costs of herbicide applications. Fisk et al. (2001) found that perennial weeds (dry weight) were reduced by as much as 75% following a cover crop of annual legumes during the summer fallow period. Cover crops may also serve to break the disease and pest cycles in a no-till operation. This can allow producers to reduce inputs of insecticides and fungicides, lowering their operating cost in addition to reducing the amount of these chemicals that could potentially contaminate surface and ground water bodies. In Washington state, McGuire

(2003) found that by using mustard green manures a producer could eliminate the use of fumigants to control soil-borne pests, saving approximately \$66/acre.

Legumes are commonly used as cover crops due to their abilities to fix N from the atmosphere and are expected to supply N to a following crop. Ebelhar, et al. (1984) found even with no additional N supplied to a corn crop, yields were doubled by growing a cover crop of hairy vetch (*Vicia villosa* Roth) showing that the cover crop was able to supply approximately 91 kg ha⁻¹ of N to the following corn crop (Ebelhar, et al., 1984). Yield reduction is a commonly cited reason for producers to avoid using leguminous cover crops to replace or supplement N fertilization. However, Ebelhar et al. (1984) found that year-to-year trends showed the corn yields remained consistently higher with hairy vetch treatments at N fertilizer rates of 0, 50 and 100 kg N ha⁻¹. A meta-analysis of studies evaluating legume only fertilization as compared to conventional systems using inorganic N fertilizer found that yield reductions (relative to conventional systems) only occurred in legume systems when less than 110 kg N ha⁻¹ was supplied by the legumes for corn, grain sorghum, and various vegetable crops (Tonitto, et al., 2006).

Using legumes as a cover crop to supplement inorganic N fertilization can be an effective management tool if implemented correctly. However, knowledge is needed to understand how this organic N source is released to the cash crop and to optimize utilization of this N source by the cash crop. Although volumes of research have been conducted in other regions to demonstrate decreased fertilizer N requirements for cash crops following cover crops, few have been conducted in the Southern Plains. Additionally, those that have been conducted were not designed to develop N utilization coefficients that are needed for producers to estimate N contribution based on cover crop biomass N production. In the Northern Great

Plains, Walley, et al. (2007) when reviewing available data from the region, found that the variability in N₂ fixation between legume species was too great to accurately predict N contribution to subsequent crops, especially for yearly or short-term predictions. However, it is unclear whether the studies reviewed applied N fertilizer to the legume crops. Without the ability to predict the N contribution from legume crops producers are likely to become frustrated with the practice, causing them to abandon it shortly after adoption.

Legume cover crops as summer forage for cattle were studied in central Oklahoma and were found to be a viable option for producers, although biomass produced varied from year to year with the environmental conditions (Rao and Northup, 2009). Cultivars of pigeon pea (*Cajanus cajan* L.), guar (*Cyamopsis tetragonoloba* L.), cowpea (*Vigna unguiculata* L.), and mung bean (*Vigna radiata* L.) were evaluated, and grain soybean (*Glycine max* L.) was used as a control. Short season species (mung bean, cowpea) showed an initial decline in N concentration in the biomass for the first half of the growing season, but increased as pods developed towards the end of the growing season. Long season species (guar, pigeon pea) showed a continual decline in N concentration throughout the growing season. Soybean N concentrations remained fairly constant. In vitro digestible dry matter was measured for each legume and seems to indicate the speed at which the N contained in the biomass would be released into the soil. Species with higher IVDDM, such as cowpea, mung bean, and guar would be readily broken down and therefore more rapidly available to the following crop. Pigeon pea IVDDM was lower which indicates that it is more resistant to decomposition and would require more time for the N to become available.

Using a mixture or “cocktail” of cover crops can be an effective management practice to help boost some of the beneficial properties of various cover crops. Planting a mixture of multiple

species will allow the producer to reap the benefits of each species as individual species may lack some component the producer is looking for. For example, sunn hemp (*Crotalaria juncea*) is capable of producing 134 kg N ha⁻¹ in just 2 to 3 months and shows the ability to control nematodes however has poor forage quality and seeds are expensive. But if mixed with another cover crop such as white clover (*Trifolium repens*), which does not perform well for controlling nematodes but proves excellent forage then the strengths of one crop are able to complement the weaknesses of the other. By increasing the diversity of the cover crops the benefits are able to be combined in order to provide the best results for the producer (Clark, 2007).

The use of cover crops has been demonstrated in many locations to benefit the soil and production system, through improving nutrient cycling or reducing crop pests or suppressing weed growth. Yet the majority of cover crop research has been performed in areas with very different climates and soil types from Oklahoma, such as Michigan, Washington, Kentucky and even as far north as Canada; very little research has been done for Oklahoma and the Central Great Plains region. In order for producers to expend their resources on cover crops there must be research that is relevant to their climatic conditions and needs.

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CHAPTER II

NITROUS OXIDE EMISSIONS FROM BIOFUEL FEEDSTOCKS PRODUCTION

ABSTRACT

Cellulosic biofuel has been promoted as a renewable fuel source that may reduce greenhouse gas (GHG) emissions as compared to the combustion of fossil fuels. Nitrous oxide emissions are an important component of the GHG lifecycle for any agricultural crop, including cellulosic biofuel. Yet, there is little data available to evaluate N₂O emissions from biofuel feedstock production such as forage sorghum and switchgrass. This data is needed to accurately determine how utilization of this fuel source will impact anthropogenic GHG emissions. Therefore, a study was conducted in Stillwater, OK to measure N₂O emissions from the potential biofuel feedstocks; forage sorghum, switchgrass, and mixed grasses. Biomass yields were different across years due to different growing conditions year to year. Cumulative N₂O emissions from the sorghum were highest in the 250 kg N ha⁻¹ N rate treatments and lowest from the 0 kg N ha⁻¹ treatments across years. The cumulative emissions from the grasses and sorghum at the 84 kg N ha⁻¹ N rate were the same. Emissions of N₂O were greatest following N fertilizer application. Under drought conditions, post-N application loss resulted in the only emission event for the year and that event was larger than events in the other 2 years.

Average cumulative N₂O losses for the 3 years was 0.75%, lower than the 1.25% estimated by the International Panel on Climate Change (IPCC). This suggests that dry land biofuel feedstock production in the southern Great Plains generates lower than estimated N₂O emissions, however yields are influenced by environmental conditions.

INTRODUCTION

The increase in anthropogenic N in the biosphere has led to an increase in atmospheric nitrous oxide (N₂O), of 12% to 23% since industrialization (Leuenberger, 1992).

Although the atmospheric concentration is relatively low (0.32 ppm), N₂O is of particular concern as the global warming potential of this gas is 310 times that of CO₂ (USEPA, 2012). In addition, N₂O has become the primary ozone depleting substance emitted by anthropogenic means (Ravishankara, et al., 2009).

Agricultural soil management in the U.S. is responsible for 69% of the N₂O emissions, this represents 3% of all GHG's emitted in the U.S. (USEPA, 2012). Fertilizer induced N₂O emissions (the difference between fertilized and unfertilized plots) are estimated to be 1.25% \pm 1.0% of N applied to agricultural fields, while indirect additions of atmospheric N₂O as a result of leaching, runoff, NO_x and NH₃ volatilization are estimated to be approximately 0.75% of N applied (Bouwman, 1996; Mosier, et al., 1996). When the concentration of inorganic nitrogen (NO₃ and NH₄) is increased through applying commercial fertilizers or mineralization of organic N sources (manure or cover crop legumes), N₂O emissions are increased above ambient levels. Many factors influence the emission of N₂O such as soil moisture, temperature, microbial activity, aeration, and organic matter content.

Mosier and Hutchinson (1981) found that N₂O emissions from a furrow-irrigated corn (*Zea mays* L.) field in Colorado were approximately 1.3% of the 200 kg N ha⁻¹ applied or 2.5 kg N ha⁻¹ for the period of time between mid-May and mid-September. Thirty percent of the N₂O emitted came during the 2 weeks following fertilization as the NH₃ from the fertilizer was undergoing nitrification. The first irrigation event for the field accounted for an additional 59% of the N₂O emitted. The authors suggested that the irrigation event reduced soil oxygen levels thereby providing for a favorable environment for denitrification. The remainder of N₂O emissions occurred rapidly following precipitation or irrigation events greater than 0.7 cm although the emissions from these events were much smaller. Soil water content was strongly correlated to N₂O emissions yet NO₃ in the soil was not since high NO₃ concentrations in soil alone do not cause denitrification to occur.

Despite the availability of research evaluating N₂O emissions from grain crop production systems, there is little data available to evaluate N₂O emissions from biofuel feedstock production such as forage sorghum and switchgrass. Crutzen, et al. (2008), by reviewing other studies, determined that common agricultural biofuel feedstocks such as soybeans, rapeseed and corn could increase climate change due to fertilizer induced emissions of N₂O that are potentially more than double (3% to 5%) the current estimates of 1.25%. The authors went on to say that N₂O emissions from biofuels such as biodiesel from soybeans or rapeseed, or ethanol from corn may offset their benefits to atmospheric greenhouse gas concentrations resulting from reduced fossil fuel combustions. Furthermore, in an effort to develop a lifecycle analysis of cellulosic and corn base ethanol production systems Farrell, et al. (2006) found that the largest single source of

uncertainty was the N₂O emissions factor due to a lack of measured emissions data and the magnitude of its influence on the lifecycle analyses. Therefore, data evaluating N₂O emissions from cellulosic biofuel feedstocks are needed in order to provide accurate life cycle analyses, thereby being able to appropriately accredit greenhouse gas offsets to production systems. Therefore, the objective of this study was to measure N₂O emissions from the potential biofuel feedstocks; forage sorghum, switchgrass, and mixed grasses. The hypotheses for this experiment was that N₂O emissions would increase with increasing N fertilization rates and that species selection would influence N₂O emissions.

MATERIALS AND METHODS

In July of 2010 experimental plots were established at Efaw Farm, Stillwater, OK on an Easpor loam (fine-loamy, mixed, superactive, thermic fluventic haplustoll). The field was previously planted to alfalfa (*Medicago sativa*). The alfalfa was terminated with cultivation approximately 12 months prior to establishment of the current study.

Switchgrass (*Panicum virgatum*), forage sorghum (*Sorghum bicolor*) and mixed grass plots of 50% switchgrass, 25% indian grass (*Sorghastrum nutans*) and 25% big bluestem (*Andropogon gerardii*) were established in a split plot design with three replications.

Each whole plot was planted to one of the three aforementioned crops and then divided into 5 subplots based on N fertilization rate. Nitrogen fertilizer applied as UAN (28-0-0) was applied to the subplots at rates of 0, 84, 168 and 252 kg ha⁻¹. The fifth subplot (legume fertilized) was not included in this study. Nitrogen fertilizer treatments were applied to forage sorghum at the 4 leaf growth stage and perennial grass treatments when first green stems appeared (Table 1). Biomass was harvested following the first killing frost with a John Deere 630 moco pull type swather (Deere and Company, Moline, IL,

USA) and baled with a John Deere 568 round baler (Deere and Company, Moline, IL, USA). The bales were then weighed individually.

Soil cores (0-40 cm) were taken from each plot in March 2010, prior to establishment of the experiment. Soil cores (0-110 cm) were again taken in March 2012, and March 2013. Soil samples were extracted with $2 \text{ mol}^{-1} \text{ L KCl}$ (1:10 soil/KCl) and analyzed for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ using flow injection analysis (QuickChem FIA+, Lachat Instruments, Milwaukee, WI).

Nitrous oxide emissions were measured using the vented chamber method as described by Mosier, et al. (1991) with base anchors measuring 38.1 cm by 12.7 cm. Chamber lids were constructed of steel and painted silver to reflect solar radiation and minimize temperature fluctuation within the chamber. Base anchors were forced into the soil so as to minimize soil disturbance within and around the anchor. In the sorghum plots, the anchors were installed across rows after planting and remained in place until the planting of the following year's crop. In the switchgrass and mixed grass plots, base anchors were installed following establishment and were not moved. Plants growing within the chambers were allowed to grow to approximately 10 cm and thereafter were kept clipped to approximately 5 cm tall.

On each sample date a vented chamber lid (7 cm x 39.4 cm x 15.2 cm) was placed into a water filled trough on the base anchor in order to form a gas tight seal with air exchange allowed through the vent tube on the lid to maintain ambient air pressure within the chamber. Gas samples of 20 mL were collected from a rubber septum in the chamber lid at 0, 15, 30 and 45 minutes following the lid being placed over the base anchor. Gas

samples were stored in 20 mL evacuated glass vials with grey rubber butyl septa until being analyzed by a gas chromatograph (Varian 450-GC) with an electron capture detector (ECD), thermoconductivity detector (TCD) and a flame ionization detector (FID) to quantify N₂O, CO₂, and CH₄, respectively. Flux measurements were taken daily for 7 days following fertilizer application, then every 7 days for the remainder of the growing season. After frost kill, sampling decreased to every 14 days until green up when sampling increased to every 7 days again. Chambers were left uncovered except during the 45 minute sampling period.

N₂O fluxes were calculated using linear regression between concentration in the chamber headspace and time. Total emissions for the growing seasons were estimated with linear extrapolation between sampling periods.

A mixed model was used for data analyses of cumulative emissions, yield, and soil NO₃-N and NH₄-N. PROC MIXED in SAS (SAS Institute, 2008) was used to test the fixed effect of N rate or species; mean separations were conducted using LSD. When comparing N rates in the sorghum, N rate was the main effect and year by N rate interactions were evaluated. At the 84 kg N ha⁻¹ rate, the main effect was species and year by species interactions were evaluated.

RESULTS AND DISCUSSION

Biomass Yields

There was a significant year by N rate interaction ($\alpha=0.05$, $p=0.0002$) for sorghum yields therefore, years were analyzed separately (Table 2). The sorghum yields for 2011 and 2012 had no significant treatment effect ($\alpha=0.05$, $p=0.1795$ and $p=0.0848$, respectively).

Sorghum yields in 2013 were significantly affected by N rate ($\alpha=0.05$, $p=0.0096$) with yields from the 0 kg N ha⁻¹ rate significantly lower than any other rate. There was no significant difference in yield among the 84, 167, or 250 kg N ha⁻¹ rates in 2013.

The yield response for the three species at the 84 kg N ha⁻¹ rate had a significant year by species interaction ($\alpha=0.05$, $p=0.0004$). Yields for the 84 kg N ha⁻¹ rate in 2011 and 2012 were not significantly different among species ($\alpha=0.05$, $p=0.9999$, $p=0.7954$, respectively). The yields among species in 2013 were significantly different ($\alpha=0.05$, $p=0.0062$), with the sorghum yield being significantly higher than switchgrass and the mixed grasses (Table 3).

In general, yields in 2011 and 2012 were negatively affected by drought. Total in season rainfall for 2011 (May-Apr) was 220 mm and total in season rainfall for 2012 (Apr-Apr) was 350 mm, in contrast the in season rainfall was 600 mm in 2013 (Apr-Nov).

N₂O Emissions

Analysis of variance found no significant interaction between N rate and year for the cumulative N₂O emission from the sorghum treatments ($\alpha=0.05$, $p=0.3246$). There was a significant difference between N rates across years with the highest cumulative N₂O emissions found in the 250 kg N ha⁻¹ rate and the lowest emissions from the 0 kg N ha⁻¹ plots (Table 4). There was no significant difference between the 84 and 167 kg N ha⁻¹ rates. Analysis of variance found no significant difference in mean cumulative N₂O emissions between years across N rates for the sorghum plots despite 2013 having almost half the cumulative emissions of the previous 2 years. (Table 4).

Analysis of variance found no interaction between species and year for the cumulative N₂O emissions for the three species at the 84 kg N ha⁻¹ rate and no significant differences between years or species (Table 5).

Cumulative emissions from the sorghum plots for the 3 year period measured followed a general linear trend increasing with N rate ($R^2=0.93$) (Figure 1), similar to the linear trends found by Dobbie, et al. (1999) for winter wheat, potatoes, broccoli, rape, and grasslands, Dusenbury, et al. (2008) for conventional tillage wheat-fallow, no-till (NT) wheat-fallow, NT wheat-wheat, and NT wheat-pea (*Pisum sativum*), and van Groenigen, et al. (2004) for silage corn. The results from Kaiser, et al. (1998) are similar to the results of this study, where the highest emissions were found in the high (250 kg N ha⁻¹) rate and the lowest emissions were from the unfertilized (0 kg N kg⁻¹) and the mid-range N rates (84 and 167 kg N ha⁻¹) were not significantly different from each other (Table 4).

In 2011, the largest flux event occurred during a three day period directly after fertilization which occurred on 23 May (Figure 2). The N₂O emissions observed during this event accounted for approximately 30% of the cumulative N₂O emissions from the fertilized treatments during the 2011 measurement period and were the largest fluxes measured throughout the three year study. For example, the N₂O flux of 2.95 mg N₂O m⁻² measured on 26 May is 4.9 times larger than any other event that occurred throughout the remainder of the three year study. This explains why the cumulative emissions observed in 2011 were not significantly different than those observed in 2012 and 2013 despite the lower rainfall observed during this growing season (Figure 5).

In 2012, the peak N₂O flux occurred approximately six weeks (41 DST) following N application (Figure 3) and were stimulated by multiple small (10-15 mm) rainfall events (Figure 5). At approximately 10 weeks (75 DST) a 30 mm rain event generated a large flux event and at approximately 20 weeks (140 DST) a third large flux event was prompted by a 50 mm rainfall. During the 2013 crop year, N₂O flux peaked again at approximately six weeks following N application, but subsided after 13 weeks with fluxes after this time being only slightly above detection limits (Figure 4). This is consistent with the findings of Dusenbury, et al. (2008) and van Groenigen, et al. (2004). Dusenbury, et al. (2008) found elevated emissions began within a week following N fertilization typically peaked after two to four weeks, and continued to have elevated (above background) emissions for approximately 10 weeks. Furthermore, a delayed period of N₂O flux up to 17-21 weeks following N application to a clay soil was described by van Groenigen, et al. (2004) and was associated with relatively wet conditions. Kaiser, et al. (1998) noted high temporal variability in N₂O emissions as a result of environmental conditions and timing of N fertilization, and this study supports that conclusion. It is useful to note that rainfall of 600 mm for the 2013 growing season was the highest of all three years, despite this greater rainfall, N₂O emissions were lowest during the 2013 measurement period.

The growing season for 2013 was the most favorable of the three years measured, due to timely rainfall (Figure 5); this resulted in the largest yields and the smallest N₂O emissions of the three years when averaged across N rates for the sorghum species. It is likely that the large amounts of biomass produced in 2013 lowered emissions as a result

of N uptake into the biomass which reduced the amount of $\text{NO}_3\text{-N}$ in the soil available to the denitrification process.

Nitrogen losses as $\text{N}_2\text{O-N}$ averaged 0.75% of applied N for the three years; these losses are lower than other reported values of 0.8% to 4.5%, as well as being lower than the IPCC estimate of 1.25% (De Klein, et al., 2006; Dusenbury, et al., 2008; van Groenigen, et al., 2004). The wide range of reported N_2O losses from N fertilizer indicates the large degree of variability in N_2O emissions and therefore the difficulty of accurately predicting emissions.

Soil NO_3 and NH_4

Analysis of variance for $\text{NO}_3\text{-N}$ concentration in the sorghum plots found no significant interaction between N rate and year, and the main effect of N rate was not significant at any soil depth, where depth was treated as a repeated measure. Year was significant ($\alpha=0.05$, $p=0.0479$) at the 0-10 cm soil depth. Soil $\text{NO}_3\text{-N}$ was significantly higher in 2012 than in 2010 or 2013 (Table 6). There was no species by year interaction and year nor was there a significant species effect on soil $\text{NO}_3\text{-N}$ concentrations. Table 7 shows the $\text{NO}_3\text{-N}$ concentrations found in each year of the study, which had no significant differences in soil $\text{NO}_3\text{-N}$ between years.

Analysis of variance for $\text{NH}_4\text{-N}$ concentration in the sorghum plots found no significant interaction between N rate and year at any soil depth and no significant effect of year or N rate (Table 8). In the 84 kg N ha^{-1} rate, there was no significant interaction between species and year at any soil depth for soil $\text{NH}_4\text{-N}$ concentration. At the 0-10 cm soil depth species was found to be significant. In the 0-10 cm soil depth, sorghum had the lowest

NH₄-N concentration and was significantly lower than the mixed grasses but was not significantly different from switchgrass (Table 9). The lack of differences in the inorganic N observed in the spring of 2010, 2012, and 2013 among N rate treatments for the sorghum species suggest that there was no significant accumulation of inorganic N in the fertilized treatments despite the fact that yields were not increased with N rates above 84 kg N ha⁻¹. In contrast, the N₂O emissions were linearly proportional to N application rate and N₂O emissions were elevated above the check throughout the growing season in the 2012 and 2013 crop years. Kaiser, et al. (1998) reported a correlation between soil NO₃ content and N₂O emissions only during the vegetative period of the study. In contrast, the lack of difference in the mineral N concentration found in soil samples collected prior to fertilization indicate that residual N is not a good indicator of N₂O emissions due to the dynamic nature of soil mineral N content and the immediate influence of fertilizer applications. Furthermore, the elevated NH₄-N found in the mixed grass treatment did not increase N₂O emissions from this treatment as indicated by no difference in N₂O emissions among the three species.

CONCLUSIONS

Nitrous oxide emissions were influenced by N rate. Cumulative N₂O emissions from the biofuel sorghum were highest from the 250 kg N ha⁻¹ rate and lowest from the 0 kg N ha⁻¹ rate. There was no difference in N₂O emissions between the middle rates (84 and 167 kg N ha⁻¹) and there was no yield difference between the 84, 167, and 250 kg N ha⁻¹ rates. The lack of yield difference paired with the reduced N₂O emissions may indicate ideal N fertilization rates for sorghum grown for biofuel feedstocks while reducing GHG emissions. The three year average N₂O loss from this study was 0.75%, of applied N

which is approximately half of the current estimated of 1.25% from the IPCC. This illustrates the variability in N₂O measurements especially in semiarid regions, and the need for more data to be included from semiarid regions in order to more accurately estimate N₂O losses from N fertilizer applications.

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Table 1. Nitrogen fertilization dates for perennial grass crops and sorghum crops for each

Cropping System	Perennial Grasses	Sorghum
Year	Fertilization Date	
2010	9 July	9 July
2011	23 May	23 May
2012	19 April	4 May
2013	30 April	7 June

year since establishment.

Table 2. Mean biomass yields for sorghum crop year by nitrogen (N) rate (kg ha⁻¹).

Year	2011	2012	2013
N Rate	Mg ha⁻¹		
kg N ha ⁻¹			
0	6.65 ^{a†}	10.97 ^{a†}	17.19 ^b
84	4.39 ^a	12.85 ^a	32.77 ^a
167	2.92 ^a	15.90 ^a	30.68 ^a
250	3.50 ^a	12.23 ^a	29.92 ^a

†Within columns, means followed by the same letter are not significantly different according to LSD ($\alpha=0.05$).

Table 3. Mean biomass yields for each species by crop year for the 84 kg N ha⁻¹ rate.

Year	2011	2012	2013
Species	Mg ha⁻¹		
Switchgrass	4.37 ^{a†}	13.18 ^{a†}	14.47 ^b
Mixed Grass	4.39 ^a	11.78 ^a	14.59 ^b
Sorghum	4.40 ^a	12.85 ^a	32.77 ^a

†Within columns, means followed by the same letter are not significantly different according to LSD ($\alpha=0.05$).

Table 4. Mean cumulative emission (kg ha^{-1}) of nitrous oxide (N_2O) for each crop year by nitrogen (kg N ha^{-1}) rate.

Year	2011	2012	2013	3 Year Average
N Rate	kg $\text{N}_2\text{O ha}^{-1}$			
kg N ha^{-1}				
0	0.73	0.67	0.47	0.61 ^{c†}
84	1.80	2.26	1.67	1.89 ^b
167	2.36	2.20	1.77	2.11 ^b
250	4.83	4.36	2.29	3.85 ^a
Annual Average	2.43	2.37	1.54	

†Within columns, means followed by the same letter are not significantly different according to LSD ($\alpha=0.05$).

Table 5. Mean cumulative emission (kg ha^{-1}) of nitrous oxide (N_2O) for each crop year by species for the 84 kg ha^{-1} nitrogen (N) rate.

Year	2011	2012	2013	3 Year Average
Species	kg $\text{N}_2\text{O ha}^{-1}$			
Switchgrass	1.08 [†]	1.50	2.55	1.71
Mixed Grasses	1.42	1.58	1.82	1.61
Sorghum	1.80	2.26	1.62	1.89
Annual Average	1.43	1.77	2.00	

†Means were not significant at the $p=0.05$ probability level.

Table 6. Mean soil nitrate concentration (mg NO₃ kg⁻¹) for each sample year for sorghum plots.

Depth	Year	2010	2012	2013
	N Rate	mg NO ₃ kg ⁻¹ soil		
cm	kg N ha ⁻¹			
0-10	0	3.07	3.88	3.62
	84	1.99	6.58	3.76
	167	3.30	7.31	3.96
	250	3.96	12.32	4.33
	Average	3.08 ^{b†}	7.52 ^a	3.73 ^b
10-20	0	4.01	3.20	2.51
	84	3.61	4.68	4.52
	167	6.62	4.08	4.14
	250	3.87	8.30	3.39
	Average	4.53 ^a	5.07 ^a	3.64 ^a
20-40	0	6.98	4.61	2.63
	84	6.24	5.51	3.95
	167	6.82	5.93	6.67
	250	6.82	10.00	3.59
	Average	6.72 ^a	6.51 ^a	4.21 ^a
40-80	0	N/A	2.35	3.47
	84	N/A	5.94	4.00
	167	N/A	6.25	6.14
	250	N/A	8.44	4.04
	Average	N/A	5.74 ^a	4.36 ^a
80-110	0	N/A	3.50	3.97
	84	N/A	5.63	2.14
	167	N/A	3.91	3.35
	250	N/A	6.48	4.80
	Average	N/A	4.73 ^a	3.42 ^a

† Within rows, means followed by the same letter are not significantly different according to LSD ($\alpha=0.05$).

Table 7. Mean soil nitrate concentration ($\text{mg NO}_3 \text{ kg}^{-1}$) for each sample year across species at the 84 kg ha^{-1} nitrogen (N) rate.

Year	2010	2012	2013
Depth			
cm	mg $\text{NO}_3 \text{ kg}^{-1}$ soil		
0-10	2.31 [†]	5.28	3.76
10-20	5.20	4.18	3.89
20-40	7.41	5.41	5.91
40-80	N/A	4.21	3.84
80-110	N/A	5.19	2.12

[†]Means were not significant at the $p=0.05$ probability level.

Table 8. Mean soil ammonium concentration (mg NH₄ kg⁻¹) for each sample year for sorghum plots.

Depth cm	Year	2010	2012	2013
	N Rate kg N ha ⁻¹	mg NH ₄ kg ⁻¹ soil		
0-10	0	19.30	9.23	13.04
	84	16.23	11.25	13.01
	167	16.60	14.10	13.20
	250	20.07	9.73	12.34
	Average	18.05 [†]	11.08	12.93
10-20	0	11.93	10.22	12.50
	84	13.67	10.46	9.46
	167	12.93	10.80	12.31
	250	12.63	9.41	11.37
	Average	12.79	10.22	11.41
20-40	0	7.33	9.76	9.41
	84	6.54	13.77	9.23
	167	8.02	14.15	9.46
	250	7.13	17.00	9.31
	Average	7.26	13.67	9.35
40-80	0	N/A	8.85	7.73
	84	N/A	12.81	21.00
	167	N/A	16.11	22.76
	250	N/A	9.39	7.17
	Average	N/A	10.73	13.70
80-110	0	N/A	9.20	8.34
	84	N/A	11.69	12.10
	167	N/A	13.95	10.78
	250	N/A	13.05	9.04
	Average	N/A	12.13	10.07

[†]Means were not significant at the p=0.05 probability level.

Table 9. Mean soil ammonium concentration ($\text{mg NH}_4 \text{ kg}^{-1}$) for each sample year across species at the 84 kg ha^{-1} nitrogen (N) rate

Species	Switchgrass	Mixed Grasses	Sorghum
Depth			
cm	$\text{mg NH}_4 \text{ kg}^{-1} \text{ soil}$		
0-10	16.14 ^{ab†}	18.29 ^a	13.73 ^b
10-20	13.45 ^a	13.95 ^a	11.19 ^a
20-40	11.54 ^a	10.65 ^a	9.85 ^a
40-80	7.64 ^a	10.57 ^a	13.03 ^a
80-110	9.00 ^a	11.66 ^a	10.94 ^a

†Within row, means followed by the same letter are not significantly different according to LSD ($\alpha=0.05$).

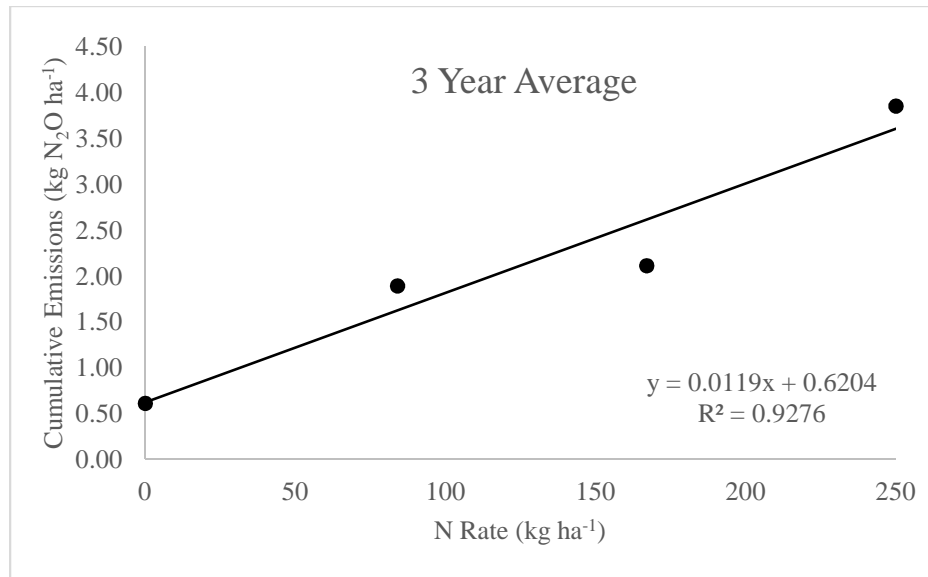


Figure 1. Three year average cumulative N₂O emissions ($\text{kg N}_2\text{O ha}^{-1}$) for each N rate. Cumulative emissions followed a linear trend.

Mean Flux by Days Since Treatment and N Rate
2011

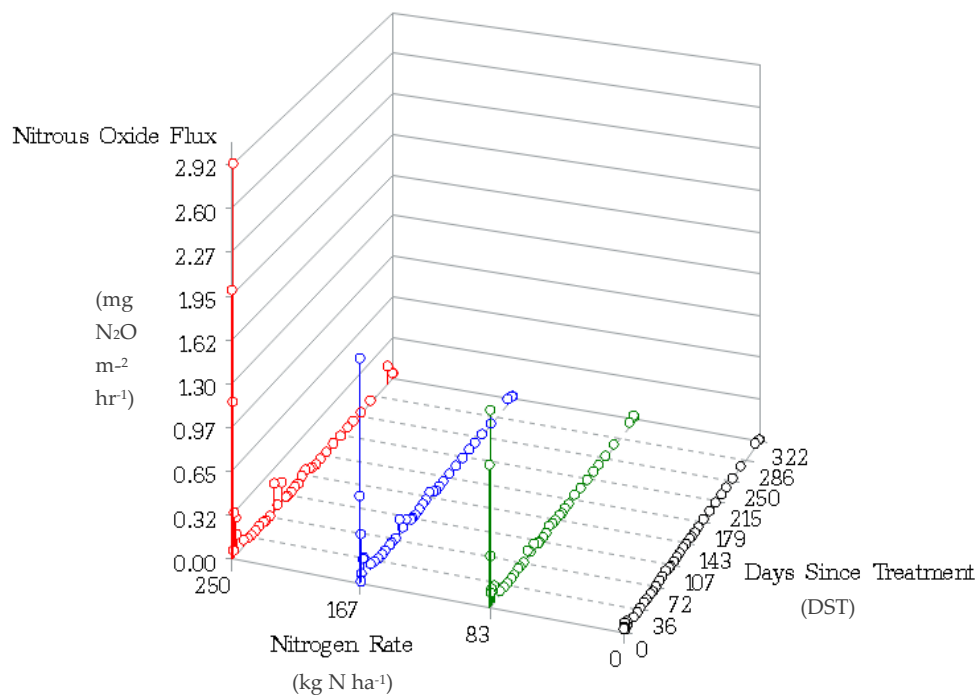


Figure 2. Mean 2011 N_2O flux by days since treatment (DST) and N fertilization rate for sorghum plots.

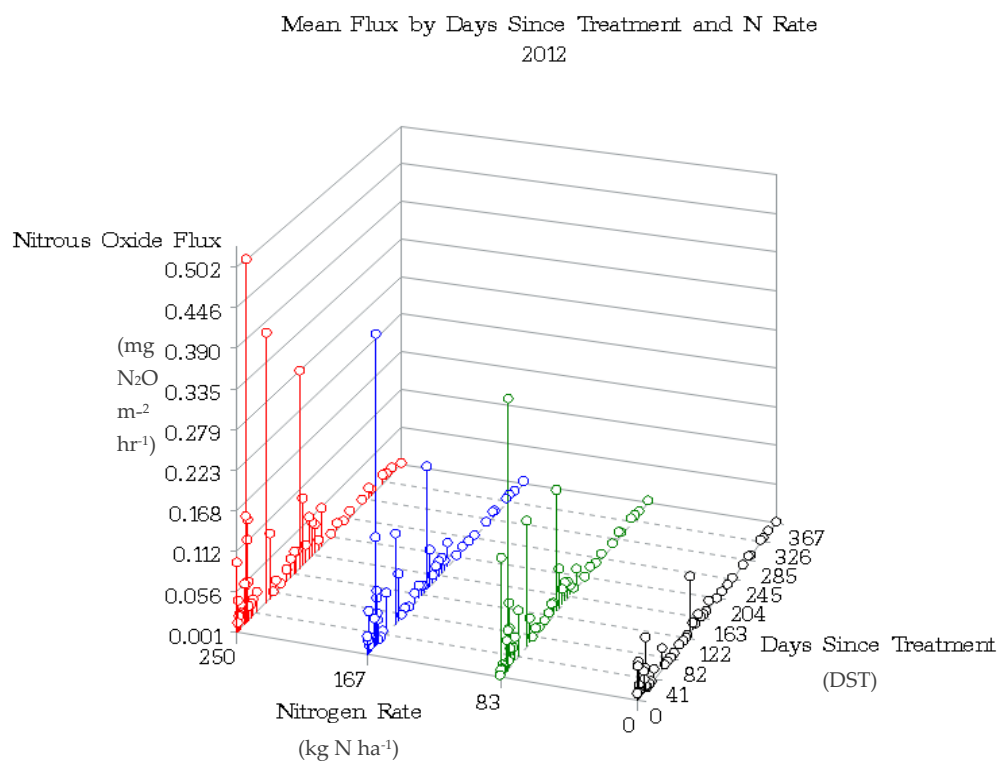


Figure 3. Mean 2012 N₂O flux by days since treatment (DST) and N fertilization rate for sorghum plots.

Mean Flux by Days Since Treatment and N Rate
2013

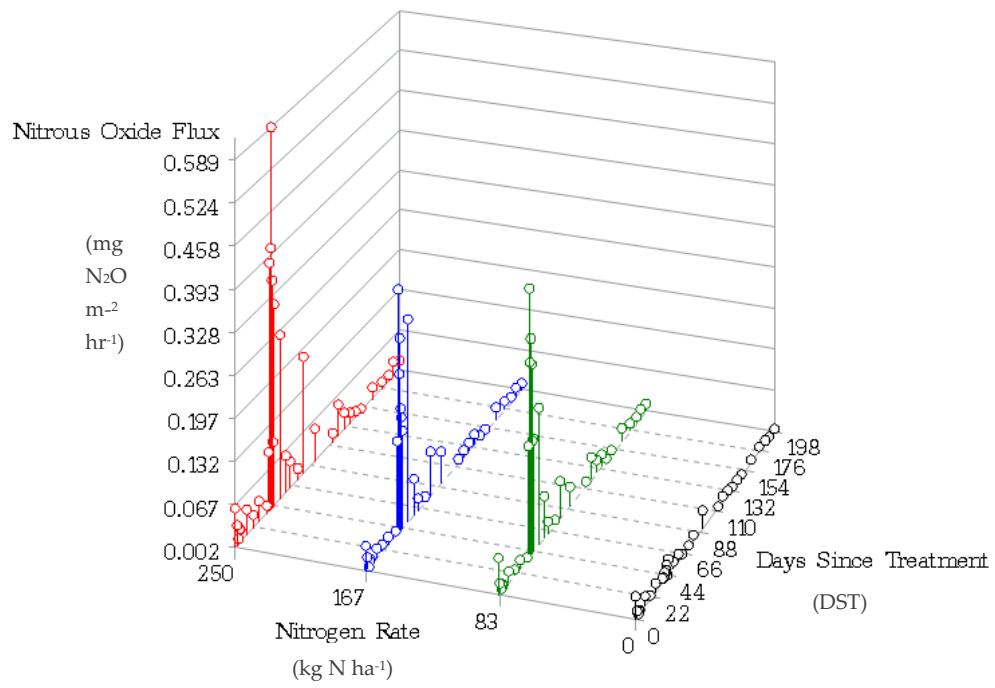


Figure 4. Mean 2013 N₂O flux by days since treatment (DST) and N fertilization rate for sorghum plots.

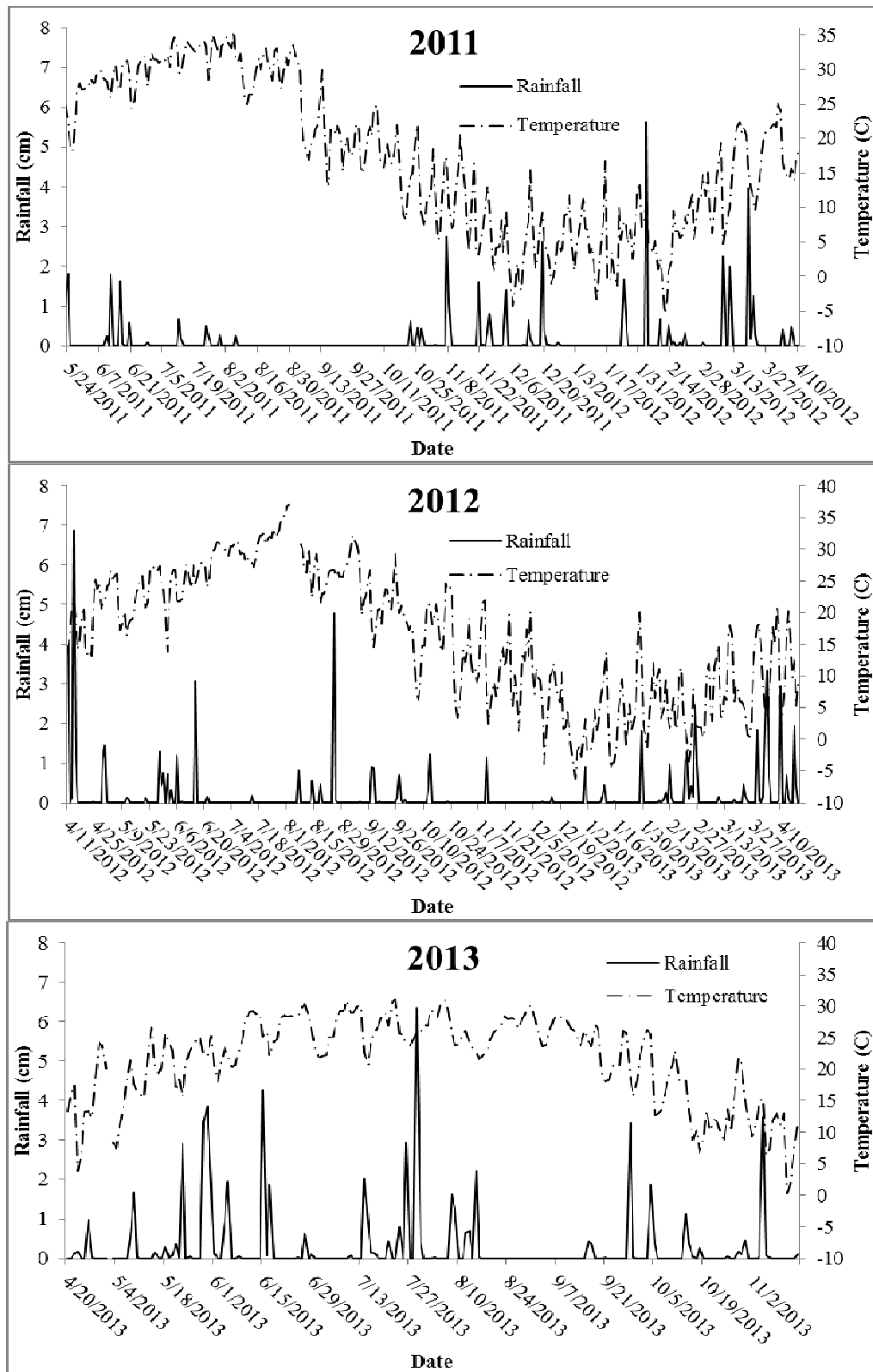


Figure 5: Average daily rainfall (cm) and average daily temperature (C) for each crop year.

CHAPTER III

NITROUS OXIDE EMISSIONS FROM WINTER WHEAT

IN THE SOUTHERN GREAT PLAINS

ABSTRACT

It is estimated that approximately 69% of the N₂O from agriculture in the United States is a result of soil management, specifically, application of synthetic nitrogen (N) fertilizers. Fertilizer induced N₂O emissions (the difference between fertilized and unfertilized soils) are estimated to be $1.25\% \pm 1.0\%$ of N applied to agricultural fields. The most reasonable approach to limiting N₂O production in soils is by improving NUE in agricultural systems. However, baseline data on the rate of emissions is needed to determine the potential impact that these efforts might have on N₂O concentrations in the atmosphere. There are few studies examining the basic effects of N fertilization on N₂O emissions from dry land winter wheat in semi-arid environments. The southern Great Plains of the U.S. has no data evaluating the impact of N application rate on N₂O emission from winter wheat. Thus, this winter wheat production area, representing 20.9 million acres is not represented within global N₂O emission estimates used by the IPCC. Therefore a study was established in a long term continuous winter wheat fertility experiment in Stillwater, OK to determine the effects of N rate on N₂O emissions from dry land winter wheat in the southern Great Plains of the U.S. in order to fill this knowledge gap.

INTRODUCTION

Emissions of nitrous oxide (N₂O) are the result of natural processes occurring in the soil. Nitrous oxide is naturally occurring greenhouse gas (GHG) that is 310 times more potent than CO₂. Therefore, relatively small emissions of N₂O into the atmosphere can have a large impact on the atmospheric greenhouse gas concentrations. Furthermore, it is estimated that approximately 69% of the N₂O from agriculture in the United States is a result of soil management, specifically application of synthetic nitrogen (N) fertilizers (USEPA, 2012). The production of N₂O in soils is a result of both nitrification and denitrification. The addition of N fertilizer increases the concentration of NO₃-N and/or NH₄-N in the soil thereby increasing the amount of N available to microbes in the soil for nitrification or denitrification and potential loss as N₂O (Bremner and Blackmer, 1978). Fertilizer induced N₂O emissions (the difference between fertilized and unfertilized soils) are estimated to be 1.25% ± 1.0% of N applied to agricultural fields (Bouwman, 1996).

In the Northern Great Plains region of the U.S. it was found that 4 different fertilized cropping systems all exhibited similar trends in N₂O emissions. The following crop systems were evaluated for N₂O emissions over a 2 year period: conventional tillage (CT) winter wheat (*Triticum Aestivum* L.) -fallow, No-Till (NT) winter wheat-fallow, NT winter wheat-spring wheat, NT winter wheat-spring pea (*Pisum sativum*), and alfalfa (*Medicago sativa* L.) -perennial grass (control). All systems except the alfalfa system were fertilized with a low (0 kg N ha⁻¹), a moderate (100 kg N ha⁻¹), and a high (200 kg N ha⁻¹) N-rate. Following fertilization all systems had N₂O emissions above the corresponding unfertilized control treatments for approximately 10 weeks in the spring and fall. Elevated N₂O flux was also measured during freeze-thaw cycles in the winter

and spring. The post-fertilization periods and the freeze-thaw cycles accounted for the 56-78% of the emissions during the 2 year study. Fertilizer induced emissions made up 37-72% of emissions with significant differences between the moderate and high rates for the CT and NT wheat-fallow and significant differences between all three rates for NT wheat-wheat and NT wheat-pea systems (Dusenbury, et al., 2008).

Since the production of N_2O in soil occurs naturally there is no method that will eliminate emissions entirely. The only proven method that will reduce N_2O emissions is to decrease N rates applied. However, the reductions in N_2O emissions from decreasing N fertilization comes at the expense of food yields. The most reasonable approach to limiting N_2O production in soils is by improving NUE in agricultural systems. However, before efforts are made to assess the impact of management on N_2O emissions can be made for a regional production system, baseline data on the rate of emissions is needed to determine the potential impact that these efforts might have on N_2O concentrations in the atmosphere.

There are few studies examining the basic effects of N fertilization on N_2O emissions from dry land winter wheat in semi-arid environments. In Germany, Kaiser and Heinemeyer (1996) found large seasonal variability in measured N_2O emissions from a sugar beet-winter wheat-winter barley rotation. The high N_2O flux rates found were measured within a week of N fertilizer application and also after a rainfall event that was sufficient to fill 50% of the soil pore volume with water. Variability between years was also found to be high. Barton, et al. (2008) found that over half of the annual emission of N_2O from dry land winter wheat in Australia occurred while the field was fallow during summer and was not affected by N fertilization. The annual emissions for the one year

measured was 0.02% of applied N which is considerably lower than the IPCC estimate of 1.25%. The authors suggest that the IPCC value may not accurately reflect the N₂O emissions from soils in a semi-arid environment as there is limited data available regarding N₂O emissions from rain-fed cropping systems in semi-arid regions. More site years of measurements would be required to determine the accuracy of the IPCC estimate for rain-fed winter wheat in semi-arid regions. The Southern Great Plains of the U.S. has no data evaluating the impact of N application rate on N₂O emission from winter wheat. Thus, this winter wheat production area, representing 20.9 million acres is not represented within global N₂O emission estimates used by the IPCC (USDA-NASS, 2014). Therefore, the objective of this study was to determine the effects of N rate on N₂O emissions from dry land winter wheat in the Southern Great Plains of the U.S. in order to provide this needed baseline data. The null hypothesis tested was that N rate has no effect on N₂O emissions, with an alternative hypothesis that N₂O emissions would increase with increasing N fertilizer application rate. In addition, the impact of residual soil profile N on N₂O emissions was also evaluated.

MATERIALS AND METHODS

In the fall of 2011, gas flux chambers were installed in an existing long term continuous winter wheat fertility trial located at the Oklahoma State University, Agronomy Research Station in Stillwater, OK on a Kirkland silt loam (fine, mixed, superactive, thermic Udertic Paleustolls). This long term trial was established in 1968 to evaluate the impact of long term application of N, P, and K on grain yield in continuous winter wheat. This location was previously managed with conventional tillage; in 2011 the location was converted to no-till. The long term trial is designed as a randomized complete block with

4 replications; four treatments from 3 replications were selected to be sampled. The treatments selected were the N rate treatments with $30.8 \text{ kg}^{-1} \text{ ha}^{-1}$ P applied as triple super phosphate (0-46-0) and $26.9 \text{ kg}^{-1} \text{ ha}^{-1}$ K applied as potassium chloride (0-0-60) applied annually prior to planting. Nitrogen was applied as urea (46-0-0) at rates of 0, 45, 90 and $134 \text{ kg}^{-1} \text{ N ha}^{-1}$; the $134 \text{ kg}^{-1} \text{ N ha}^{-1}$ N rate was split applied so that half of the N was applied prior to planting and the remaining half was applied in the spring at GS 30 (Zadoks, et al., 1974)(Table 1). Wheat was harvested using a Massey Ferguson combine with a two meter wide cutting table. Wheat grain yields were adjusted to 12.5% moisture.

Nitrous oxide emissions were measured using the vented chamber method as described by Mosier, et al. (1991) with base anchors measuring 38.1 cm by 12.7 cm. Chamber lids were constructed of steel and painted silver to reflect solar radiation and minimize temperature fluxes within the chamber. Base anchors were forced into the soil so as to minimize soil disturbance within and around the anchor. Base anchors were installed within wheat rows after planting and remained in place until the planting of the following year's crop. Wheat plants were kept clipped to the soil surface within the chambers for the duration of the growing season. At fertilization for the 2012-2013 and 2013-2014 crop years, a 61 cm by 42 cm area was covered to exclude fertilizer from the chambers designated as residual chambers such that the impact of residual N on N_2O emissions could be assessed in these long term fertility treatments.

On each sample date a vented chamber lid (7 cm x 39.4 cm x 15.2 cm) was placed into a water filled trough on the base anchor in order to form a gas tight seal with air exchange allowed through the vent tube on the lid to maintain ambient air pressure within the chamber. Gas samples of 20 mL were collected from a rubber septum in the chamber lid

at 0, 20, 40 and 60 minutes following the lid being placed over the base anchor. Gas samples were stored in 20 mL evacuated glass vials with grey rubber butyl septa until being analyzed by a gas chromatograph (Varian 450-GC) with an electron capture detector (ECD), thermoconductivity detector (TCD) and a flame ionization detector (FID) to quantify N₂O, CO₂, and CH₄, respectively. Flux measurements were taken daily for 7 days following fertilizer application, then every 7 days for the remainder of the growing season. After harvest, sampling decreased to every 14 days until green up when sampling increased to every 7 days again. Chambers were left uncovered except during the 60 minute sampling period. Surface soil samples (0-15 cm) were collected yearly prior to pre-plant fertilizer applications using hand probes.

A mixed model was used for data analyses of cumulative emissions, yield, and soil NO₃-N and NH₄-N PROC MIXED in SAS (SAS Institute, 2008) was used to test the fixed effect of N rate or species; mean separations were conducted using LSD. Interactions between N rate treatment and year were evaluated and means were across years when no interaction was found.

RESULTS AND DISCUSSION

Yield

Wheat grain yields for the 2012-2014 harvests are presented in table 2. Yield was found to have a significant interaction between year and treatment using analysis of variance ($\alpha=0.05$, $p<0.0001$), therefore years were analyzed separately. Analysis of variance found mean grain yield in 2012 to be highest in the 45 and 90 kg N ha⁻¹ rates, with the 45 and 134 kg N ha⁻¹ rates not significantly different (Table 2). The lack of significant difference

between the 45 and 134 kg N ha⁻¹ rates is not unexpected as the 134 kg N ha⁻¹ rate is split applied (50% in the fall, 50% in the spring) and a lack of spring rainfall likely prevented the spring N application from being utilized. The lowest yield for 2012 was from the 0 kg N ha⁻¹ rate. Grain yields were low in 2013 due poor stand establishment and growth resulting from drought conditions. This low yield environment resulted in no significant differences in yield between treatments that received fertilizer additions. The 0 kg N ha⁻¹ rate had significantly higher yields than all other treatments. Yields in 2014 followed similar trends to those found in 2012 with maximum yields observed at the 90 kg ha⁻¹ rate. In fact, maximum yields were similar between the 2 years with 2533 and 2458 kg ha⁻¹ produced at the 90 kg N ha⁻¹ treatment in 2012 and 2014, respectively. However, observed differences were not significantly different in 2014. The 0 kg N ha⁻¹ treatment produced a yield of 2074 kg ha⁻¹, which was 384 kg ha⁻¹ lower than the maximum yield in 2014. In contrast, the 0 kg N ha⁻¹ treatment produced 1166 kg ha⁻¹ which was 1367 less wheat grain than the maximum yield in 2012. The lack of yield response in 2014 may be due to a freeze event that occurred 15 April which could have reduced yield in the fertilized treatments compared to the 0 kg N ha⁻¹ treatment.

Soil NO₃ and NH₄

Analysis of variance found no significant interaction between year and N rate in the post-harvest surface (0-15 cm) soil samples for either NO₃ concentration or NH₄ concentration in the soil. The main effect of N rate was not significant for NO₃ or NH₄, however the main effect of year was significant for both NO₃ and NH₄ (Tables 4 and 5). Soil NO₃ concentrations were significantly higher post-harvest in 2012 and 2013 than in 2014. Soil NH₄ concentrations were significantly higher in 2013 than in 2012 or 2014. The higher

soil NO_3 and NH_4 concentrations in 2013 is expected after the extremely low wheat yields for the 2013 crop year. The low concentrations of NO_3 and NH_4 found post-harvest in 2014 also follow a high yielding wheat crop which would have been expected to deplete soil N.

N₂O Emissions

Figures 2-4 illustrate the distribution of flux events resulting from fertilization of continuous no-till wheat in the Southern Plains of the U.S. In each year the primary flux periods occurred directly after fertilizer application and again during the summer and early fall months. The duration of the initial flux event after N application ranges from 40 during the 2011-2012 and 2012-2013 crop years. The initial flux of N_2O after N application occurred for approximately 70 days during the 2013-2014 crop year. This is consistent with data collected from studies of summer crops showing that N_2O emissions is most pronounced directly after N fertilizer application (Venterea, et al., 2005). This initial flux period was followed by a period during the winter months where fluxes were near detection limit.

Emissions of N_2O in winter wheat in Canada followed a similar temporal pattern as was seen in this study, with increased emissions following fertilization, low emissions during the winter, then an increase in emissions during the late summer/early fall (Dusenbury, et al., 2008). In 2012, the post winter flux events from the fertilized treatments were first observed in May and were sporadically observed through the remainder of the fallow period with the largest events occurring at 48 weeks after fertilizer application (Oct 2012) (Figure 3). In 2013, post winter N_2O fluxes above the detection limit became

consistently observed in April and remained elevated for the 134 kg N ha⁻¹ treatment during the remainder of the fallow period but were less consistent for the other treatments (Figure 4). Similarly to 2012, the largest flux events in 2013 occurred late in the fallow period. Barton, et al. (2008) found that the largest N₂O fluxes followed the first summer rains, not the largest rainfall event which differs from the N₂O fluxes measured in this study (Figures 3-5). In 2013 the greatest fluxes of N₂O were found just after the largest rainfall event of the year (2.9 cm) (Figure 5). In 2014, the onset of consistent N₂O fluxes above the detection limit was delayed until June after which it was consistently elevated (Figure 5). It is interesting to note that in each year the maximum flux was observed in the 134 kg N ha⁻¹ and occurred 10-12 months after N fertilizer applications. This illustrates the importance of residual N on N₂O fluxes despite the fact that significant differences in inorganic soil N were not observed after harvest.

Analysis of variance found a significant year by N rate interaction for cumulative N₂O emissions ($\alpha=0.05$, $p=0.0254$). The main effects of year and N rate were both significant (Table 6). Mean cumulative N₂O emissions for 2013 were significantly higher than 2012 and 2014. The low yields can, in part, explain why N₂O emissions were largest for the 2013. As mentioned, yields were low in 2013 due to below normal rainfall during the fall months of 2012 (Figure 8) resulting in poor stand establishment (rainfall between Sept 14 and Dec 31 was 127 mm below normal). This was followed by above normal rainfall during the spring and throughout the summer fallow period, which as mentioned above allowed for elevated N₂O fluxes to be measured in April and throughout the summer months in 2013 (Figure 4). In contrast, below average rainfall and above average temperatures observed during the 2012 summer months resulted in comparably lower

N₂O emissions (Table 6). In 2014, summer temperatures and rainfall were near normal and therefore resulted in an intermediate average cumulative emissions.

The mean values for cumulative N₂O emissions for each year and N treatment is found in Table 6. Analysis of variance found a significant year by treatment interaction.

Cumulative N₂O emissions from the 0 and 45 kg N ha⁻¹ N rates to be significantly lower than the 134 kg N ha⁻¹ N rate in 2012, with no difference between the 90 kg N ha⁻¹ treatment and remaining treatments. The cumulative N₂O emissions in 2013 followed a similar pattern where the 134 kg N ha⁻¹ rate was significantly higher than all other N rates. The residual chamber (134 kg N residual) cumulative N₂O emissions were significantly lower than the 134 kg N ha⁻¹ rate, however were no different from any of the other N rates. In 2014, the 90, 134, and 134 residual kg N ha⁻¹ treatments had significantly higher cumulative N₂O emissions than the other treatments. The 134 residual treatment was not significantly different from the 90 kg N ha⁻¹ rate or the 90 residual treatment.

Figure 1 shows the linear relationship between N rate and the cumulative annual N₂O emissions. The slope of this regression equation suggests that on average 0.023 kg N₂O will be emitted per kg N applied which is in agreement with the IPCC estimate of 0.02 kg N₂O. Assessment of regression analysis resulting from each year shows that in 2012 and 2014 the slope is 0.018 and 0.015 kg N₂O per kg N applied, respectively. In contrast, the slope for 2013 was 0.037 kg N₂O per kg N applied. The N loss as N₂O in 2013 suggests that over application of N during years where yields are low can have a profound impact on average annual emissions, particularly when rainfall and temperature conditions are conducive to the production of N₂O. Furthermore, the lack of significant differences in

inorganic N concentrations among treatments suggest that soil analysis to assess residual N after harvest is not a useful indicator of the potential for N₂O emissions, despite the observation made in the residual chambers in the 134 kg N ha⁻¹ treatment showing N₂O emissions above baseline in 2014.

CONCLUSIONS

The three year average losses of 0.023 kg N₂O per kg N applied observed in no till winter wheat production in the southern Great Plains are accurately represented by the IPCC estimate of 0.02 kg N₂O. However, each year deviated from the IPCC estimate with two years falling below, and one year (2013) was over the estimate. This variability demonstrates the dynamic nature of N₂O emissions. The primary periods of N₂O flux were following N fertilization, yet then again in the late summer and early fall months as the environmental conditions became more favorable to production of N₂O in the soil. Cumulative emissions of N₂O were highest in 2013 when wheat yields were poor, indicating a lack of crop uptake and therefore more N in soil that was available to be lost as N₂O.

Emissions of N₂O from the residual chambers containing soil that received no fertilization for 1 crop year following yearly N applications of 134 kg N ha⁻¹ produced as much N₂O as the 45 and 90 kg N ha⁻¹ treatments. This indicates that when soils have been historically fertilized at high N rates there is still potential to produce emissions of N₂O that are comparable to mid-range N fertilization. This shows that the lack of N application for these soils will not reduce production of N₂O to the same level as what is naturally produced in a historically unfertilized soil.

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Table 1. Fertilization, planting, and harvest dates for the three years measured.

Year	Fertilization	Planting	Harvest
2012	15 Sep 2011 (7 Mar 2012)*	13 Oct 2011	8 Jun 2012
2013	26 Sept 2012 (11 Mar 2013)	16 Nov 2012	28 Jun 2013
2014	10 Oct 2013 (21 Mar 2014)	22 Oct 2013	18 Jun 2014

*Date in parentheses indicates date for split application of 134 kg N ha⁻¹ rate.

Table 2. Mean wheat yield (kg ha⁻¹) for each year and N rate.

Year	2012	2013	2014
N Rate			
kg N ha ⁻¹		kg ha ⁻¹	
0	1166c†	952a	2074a
45	2238ab	618a	2363a
90	2533a	634a	2458a
134	2040b	606a	2389a

†Within columns, values followed by the same letter are not significantly different (LSD, $\alpha=0.05$).

Table 4. Post-harvest mean soil nitrate concentration ($\text{mg NO}_3 \text{ kg}^{-1}$) in 0-15 cm for each year and N rate with yearly mean and N rate mean.

Year	2012	2013	2014	3 Year Average
N Rate kg N ha^{-1}	$\text{mg NO}_3 \text{ kg}^{-1}$			
0	26.72	22.30	10.42	19.81
45	30.48	29.02	12.09	23.86
90	27.15	23.32	12.03	20.83
134	21.59	19.40	10.29	17.09
Average	26.48 ^{a†}	23.51 ^a	11.21 ^b	

†In last row, values followed by the same letters are not significantly different (LSD, $\alpha=0.05$).

Table 5. Post-harvest mean soil ammonium concentration ($\text{mg NH}_4 \text{ kg}^{-1}$) in 0-15 cm for each year and N rate with yearly mean and N rate mean.

Year	2012	2013	2014	3 Year Average
N Rate kg N ha^{-1}	$\text{mg NH}_4 \text{ kg}^{-1}$			
0	17.18	26.01	16.59	19.93
45	22.29	31.25	18.25	23.93
90	17.39	28.95	15.38	20.57
134	14.79	22.05	14.87	17.23
Average	17.91 ^{b†}	27.06 ^a	16.27 ^b	

†In last row, values followed by the same letters are not significantly different (LSD, $\alpha=0.05$).

Table 6. Mean cumulative N₂O emissions (kg N₂O ha⁻¹) by N rate and year.

Year	2012	2013	2014
N Rate			
kg N ha ⁻¹	kg N ₂ O ha ⁻¹		
0	0.79 ^{b†}	2.17 ^c	2.06 ^{cd}
45	1.42 ^b	2.63 ^{bc}	1.98 ^{cd}
90	2.02 ^{ab}	4.27 ^b	3.01 ^{ab}
134	3.21 ^a	7.13 ^a	3.87 ^a
Average	1.86 ^A	3.98 ^B	2.58 ^A
45 Residual	-	-	1.28 ^d
90 Residual	-	-	2.63 ^{bc}
134 Residual	-	3.72 ^{bc}	3.25 ^{ab}

†Within columns, values with the same lowercase letters are not significantly different (LSD, $\alpha=0.05$).

‡In row, values followed by the same uppercase letters are not significantly different (LSD, $\alpha=0.05$).

§In last column, values followed by the same uppercase letters are not significantly different (LSD, $\alpha=0.05$).

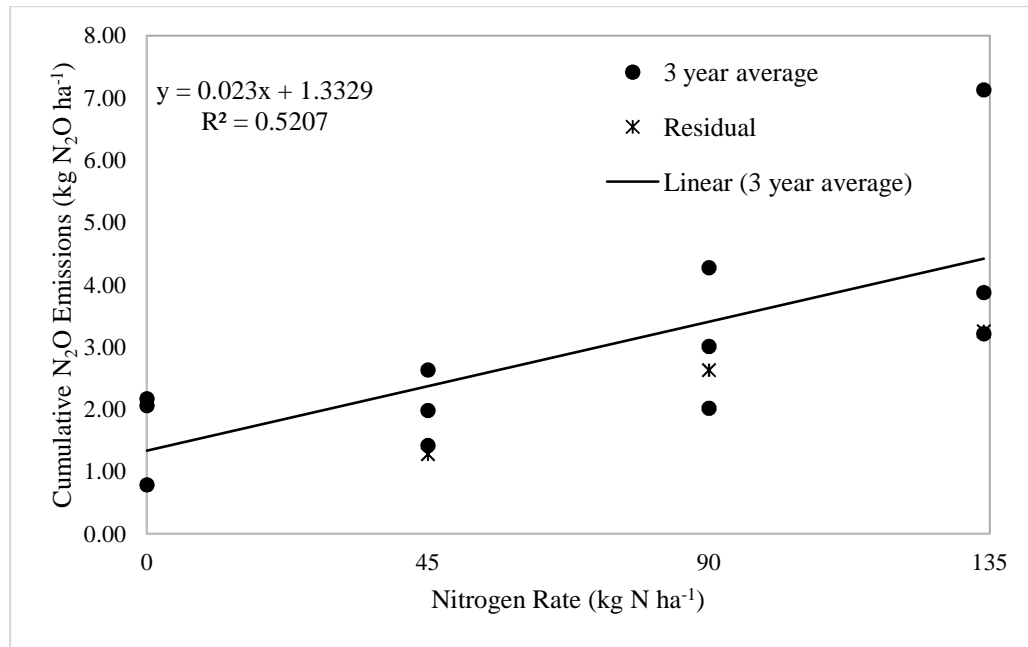


Figure 1. Three year average cumulative N₂O emissions (kg N₂O ha⁻¹) for each N rate and average cumulative emissions from residual N treatments. Cumulative emissions followed a general linear trend ($R^2=0.52$).

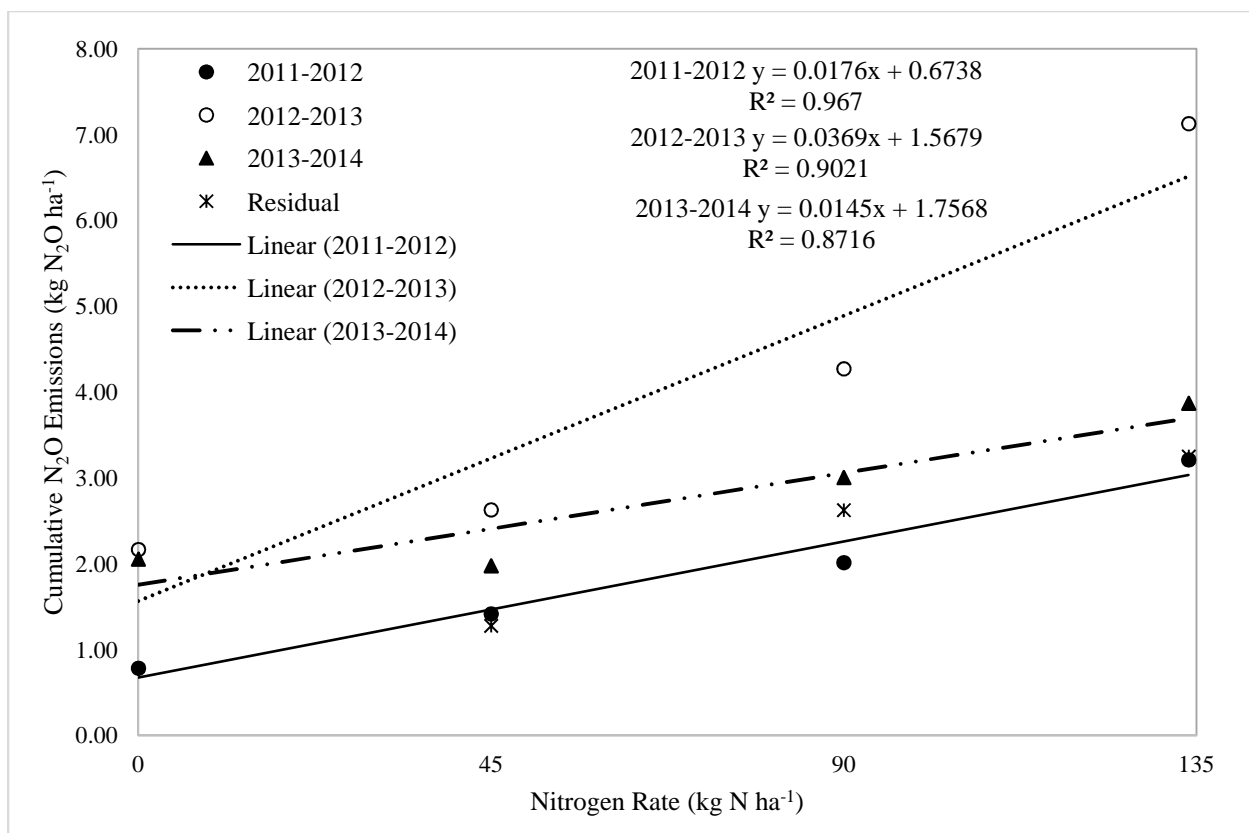


Figure 2. Cumulative N₂O emissions (kg N₂O ha⁻¹) for each year and N rate, and average cumulative emissions from residual N treatments. Cumulative emissions followed a general linear trend.

Mean Flux by Days Since Treatment and N Rate
2012

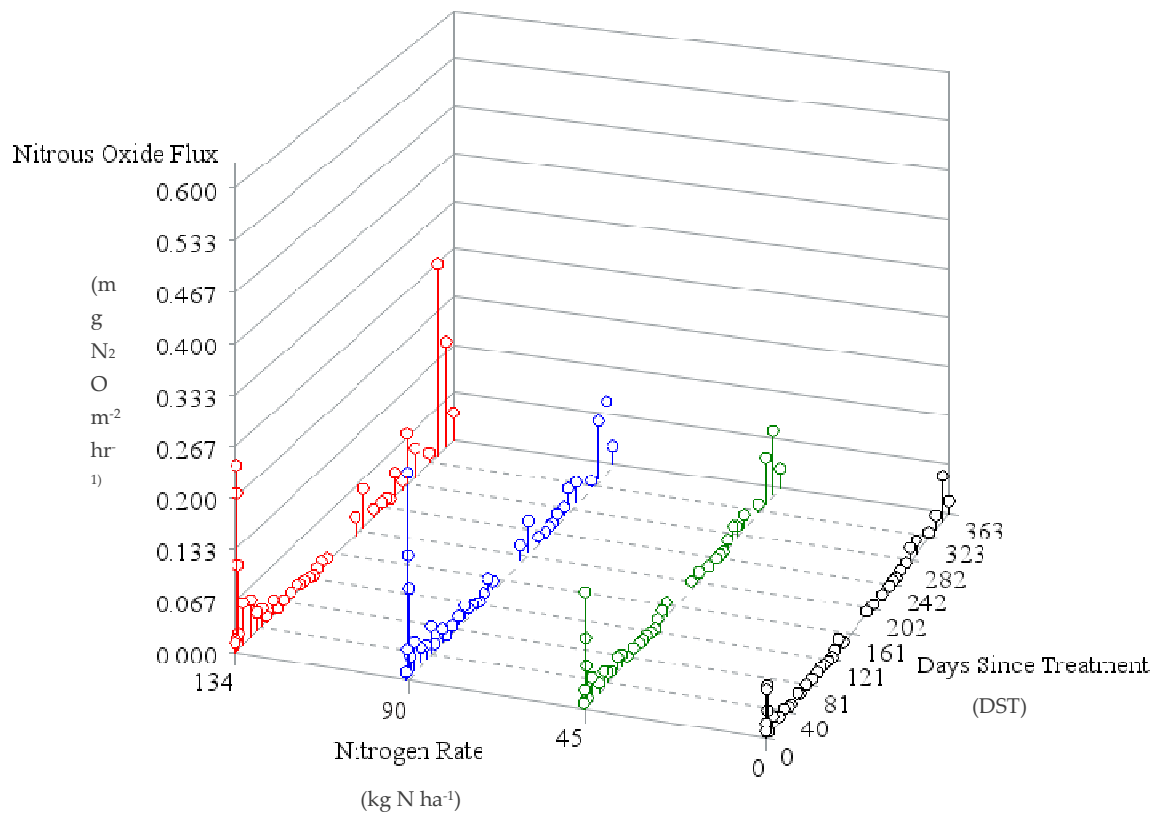


Figure 3. Mean N₂O flux for crop year 2012 for each N rate by days since N fertilization. Pre-plant N applied 15 Sept 2011 (DST=0) and top-dress application of 134 kg N ha⁻¹ rate applied 17 Mar 2012 (DST=193).

Mean Flux by Days Since Treatment and N Rate with Residuals
2013

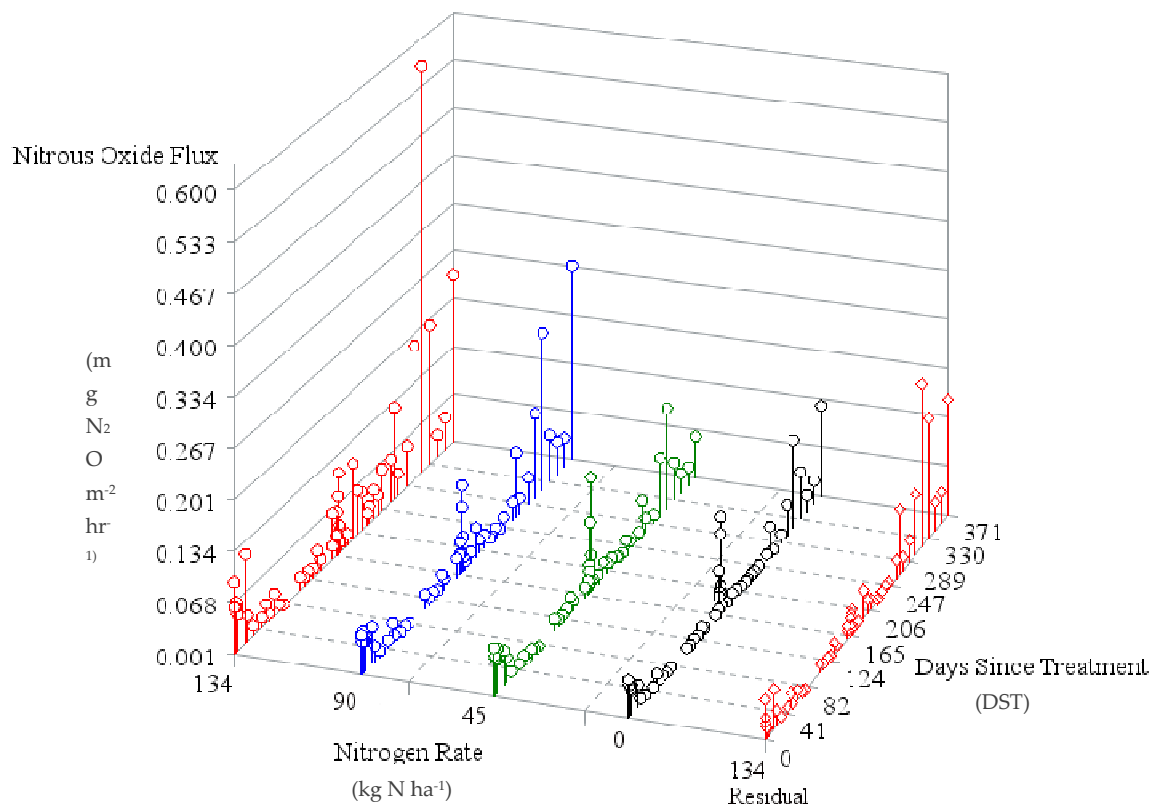


Figure 4. Mean N_2O flux for crop year 2013 for each N rate by days since N fertilization, including residual N treatments. Pre-plant N applied 26 Sept 2012 (DST=0) and top-dress application of 134 kg N ha^{-1} rate applied 11 Mar 2013 (DST=165).

Mean Flux by Days Since Treatment and N Rate with Residuals
2014

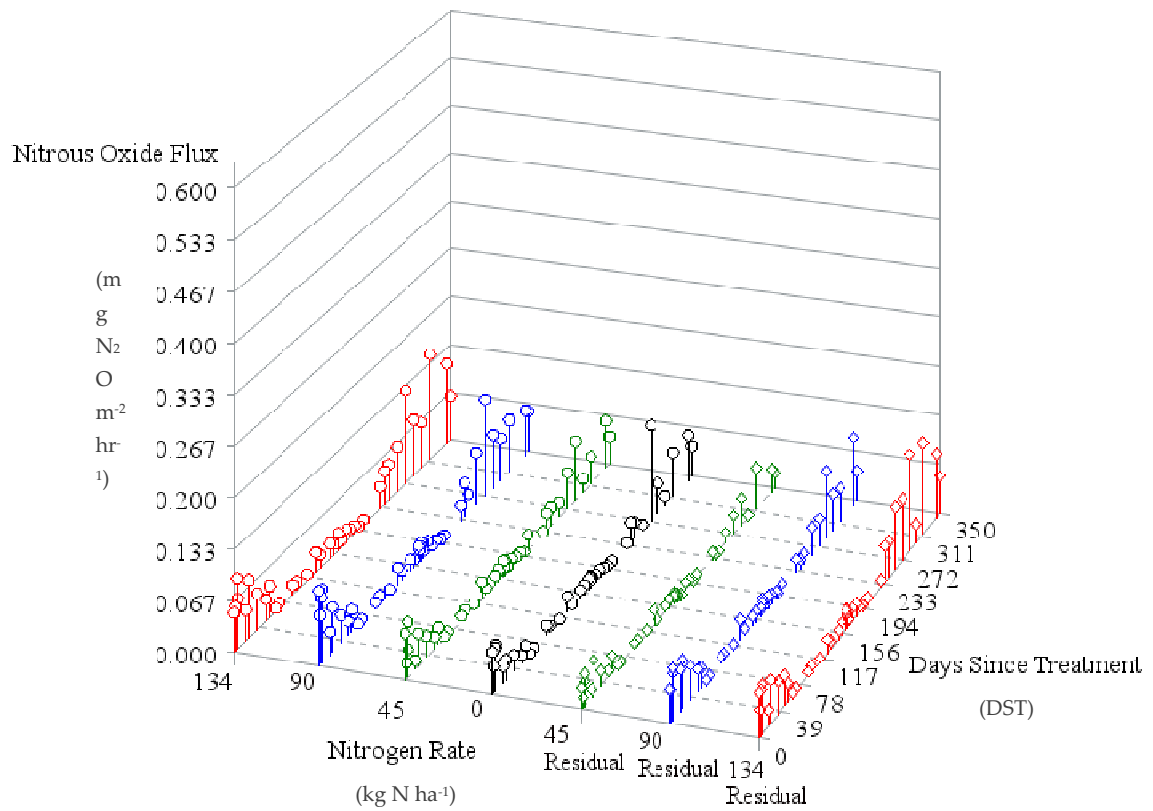


Figure 5. Mean N₂O flux for crop year 2014 for each N rate by days since N fertilization, including residual N treatments. Pre-plant N applied 10 Oct 2013 (DST=0) and top-dress application of 134 kg N ha⁻¹ rate applied 21 Mar 2014 (DST=161).

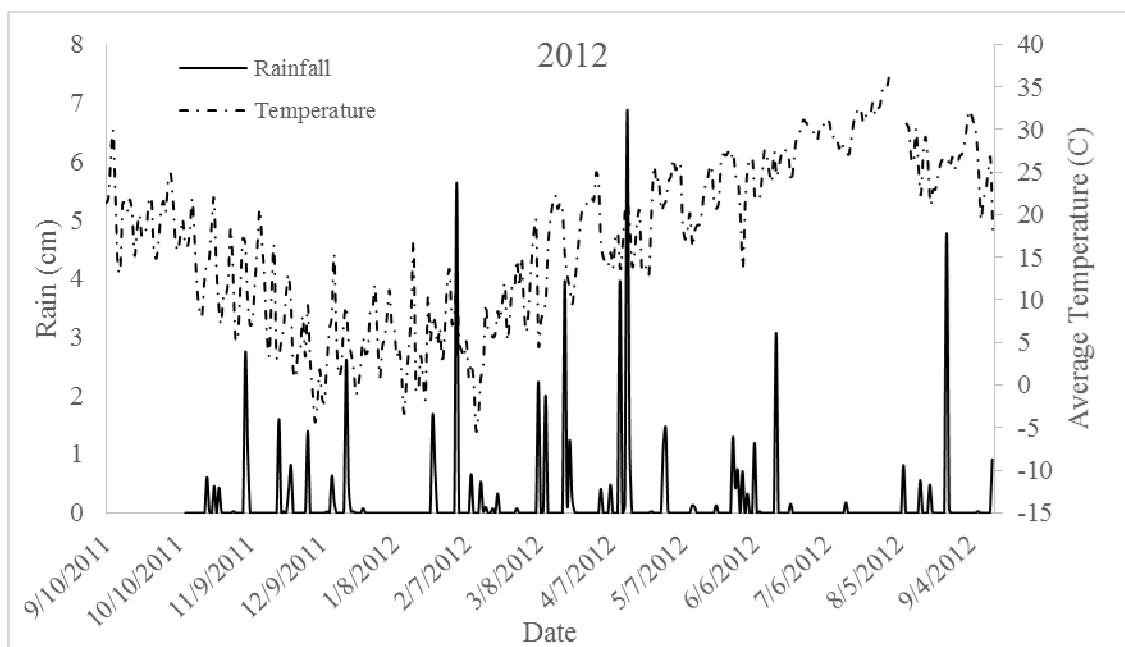


Figure 6. Average daily air temperature and total daily rainfall for the 2012 crop year. Total rainfall for 2012 crop year was 65.3 cm.

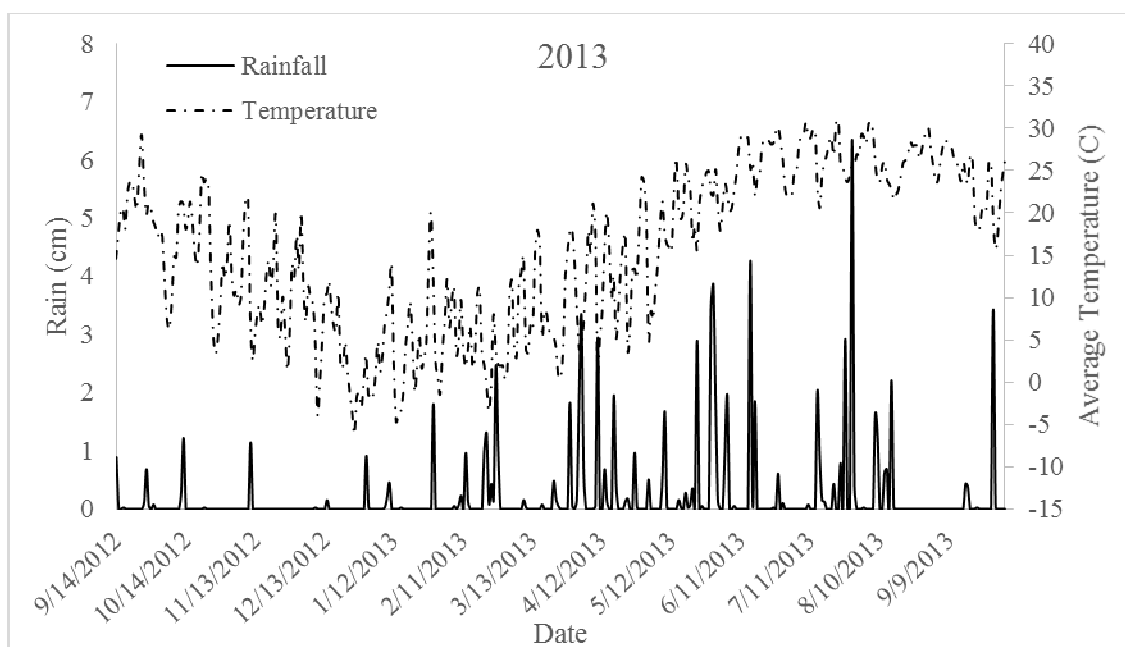


Figure 7. Average daily air temperature and total daily rainfall for the 2013 crop year. Total rainfall for 2013 crop year was 85.2 cm.

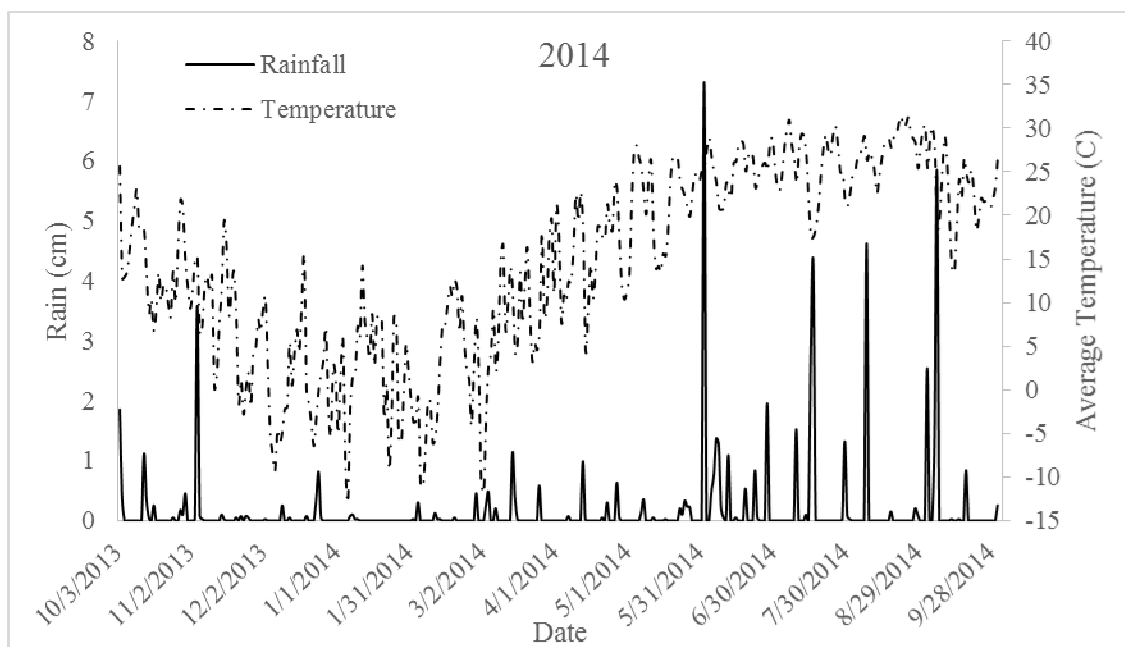


Figure 8. Average daily air temperature and total daily rainfall for the 2014 crop year. Total rainfall for 2014 crop year was 60.7 cm.

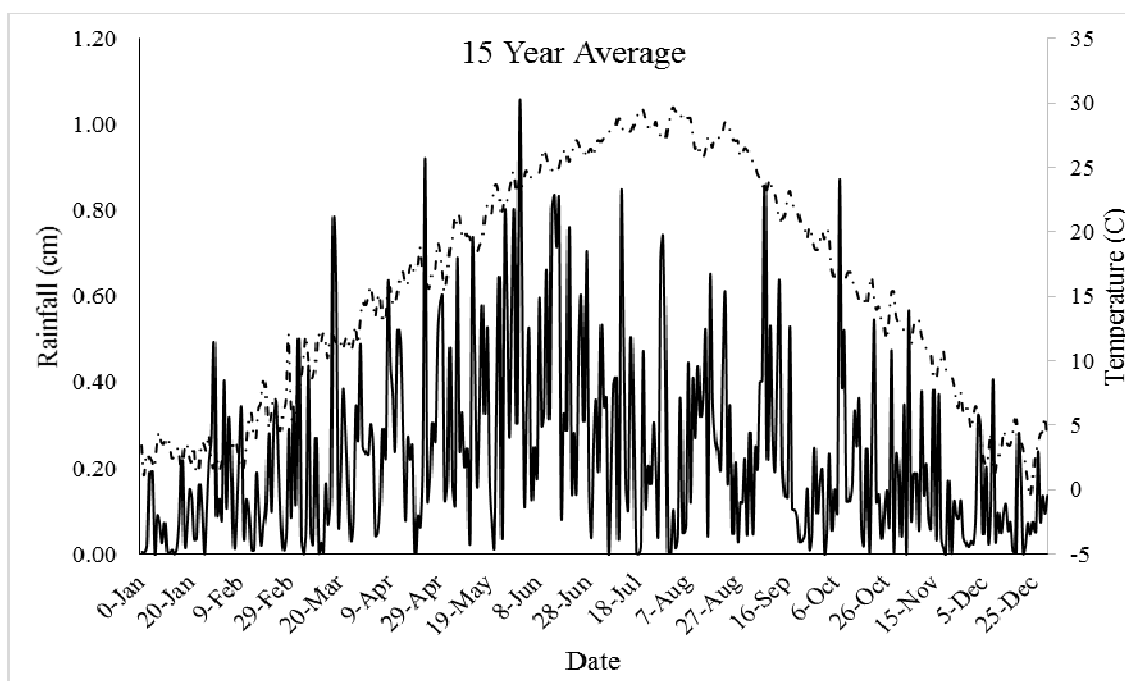


Figure 9. Fifteen year average temperature and rainfall for Stillwater, OK.

CHAPTER IV

COVER CROP MIXTURES IN WINTER WHEAT PRODUCTION

FOR THE SOUTHERN GREAT PLAINS

ABSTRACT

Cover crops have long been utilized for erosion protection and legume cover crops have been used to fix N from the atmosphere. Little research has been focused on evaluating the use of cover crop mixtures. The objectives of this study were to evaluate the impacts of using leguminous cover crop mixtures on N cycling, soil moisture, and cash crop performance in continuous no till winter wheat production. An experiment was established in Lahoma, OK in 2013 where 8 cover crop mixtures were planted and compared to traditional summer fallow treatments with N rates ranging from 36 to 136 kg ha⁻¹. Cover crops were planted in July each year following wheat harvest, terminated in August, and wheat was planted in October. No difference in wheat yield was found in 2014 following cover crops. The biomass production and biomass N content of the different cover crop mixtures was not significantly different between mixtures or years (2013 and 2014). Soil NO₃ concentration in the soil surface at wheat planting in 2014 had significant differences between cover crop mixtures. Cover crop mixtures containing grass species had the lowest soil NO₃ content, even when mixtures also contained legumes. Legume only cover crop mixtures had soil NO₃ concentrations that were no

different than the 72 kg N ha⁻¹ rate and the 136 kg N ha⁻¹ fertilizer treatments. Soil moisture was not significantly different between treatments. Incorporating summer cover crop mixtures into a continuous winter wheat system did not affect wheat yields and, when using legume only mixtures, does not deplete soil NO₃.

INTRODUCTION

Cover crops are a common tool in preventing and controlling erosion of soil in cropland. Benefits of utilizing cover crops go beyond reducing erosion to provide additional ecosystem services such as, improving water quality, weed suppression, reducing N leaching, improving soil organic matter content, fixing N (with legumes), recycling nutrients, and even potentially increasing crop yields (Winger, et al., 2012). Residue cover provided by the cover crops helps to insulate the soil and buffer it from large fluctuations in soil temperature and moisture content. By planting cover crops, the water that would be lost to evaporation during a fallow period can be put to use to produce additional residue and nitrogen when a legume cover crop is used, and to improve soil structure by maintaining an actively growing root system during what is typically the fallow period.

Legumes are popular for use as a cover crop given that they are able to fix atmospheric N, potentially reducing the need for synthetic N for the following crop. Ebelhar, et al. (1984) found that, in Kentucky, growing hairy vetch (*Vicia villosa* Roth) as a cover crop doubled corn (*Zea mays*) yields as the cover crop provided approximately 91 kg N ha⁻¹ to the following corn crop compared to an unfertilized control treatment. Year-to-year trends showed the corn yields from treatments planted to hairy vetch at N fertilizer rates of 0, 50, and 100 kg N ha⁻¹ were consistently the highest yielding treatments (Ebelhar, et

al., 1984). A meta-analysis of studies evaluating legume only fertilization as compared to conventional systems using inorganic N fertilizer found that yield reductions (relative to conventional systems) only occurred in legume systems when less than 110 kg N ha⁻¹ was supplied by the legumes for corn, grain sorghum, and various vegetable crops (Tonitto, et al., 2006).

Rao and Northup (2009) evaluated the use of warm season legume cover crops in the southern Great Plains, such as pigeon pea (*Cajanus cajan* (L.) Millsp.), guar (*Cyamopsis tetragonoloba* L.), cowpea (*Vigna unguiculata*), and mung bean (*Vigna radiata* L.) compared to the commonly used grain soybean (*Glycine max* L.) and found that biomass production varied from year to year with the environmental conditions. The short season species (mung bean, cowpea) showed an initial decline in N concentration in the biomass for the first half of the growing season, but increased as pods developed towards the end of the growing season. Long season species (guar, pigeon pea) showed a continual decline in N concentration throughout the growing season. Soybean N concentrations remained fairly constant (28.5 to 31.2 g N kg⁻¹) for the duration of the growing season. In vitro digestible dry matter (IVDDM) was measured for each legume and seems to indicate the speed at which the N contained in the biomass would be released into the soil. Species with higher IVDDM, such as cowpea, mung bean, and guar would be readily broken down and therefore more rapidly available to the following crop. Pigeon pea IVDDM was lower which indicates that it is more resistant to decomposition and would require more time for the N to become available.

Recently, using a mixture of cover crop species has gained attention as mixtures have been promoted to enhance the benefits using cover crops. By planting cover crop

mixtures, it may be possible to reap multiple benefits of cover crops in one season. For example, sunn hemp (*Crotalaria juncea*) is capable of producing 134 kg N ha⁻¹ in just 2 to 3 months and shows the ability to control nematodes however has poor forage quality and seeds are expensive. If sunn hemp is mixed with another cover crop such as white clover (*Trifolium repens*), which does not perform well for controlling nematodes but proves excellent forage then the strengths of one species are able to complement the weaknesses of the other species (Clark, 2007). By increasing the diversity of the cover crops the benefits are able to be combined in order to provide the best results for the producer (Clark, 2007). However, in Nebraska on an organic dry land sunflower (*Helianthus annuus* L.)-soybean-corn rotation, fertilized with beef manure, use of spring cover crop mixtures of two, four, six, and eight cover crop species (various legumes, buckwheat, rape, mustard, radish) resulted in no differences in soil moisture, soil N or cash crop yields (Wortman, et al., 2012).

Other regions of the US have documented the successful use of single species cover crops however, these studies tend to be dominated by cool season cover crops and are located in more humid regions of the US. Very few studies have examined the use of cover crop mixtures. Therefore, the objective of this study was to evaluate the impacts of using cover crop mixtures containing legumes on N cycling, soil moisture, and cash crop performance in continuous no till winter wheat production. The null hypothesis for this experiment was that there would be no impact of planting cover crop mixtures on soil N and soil moisture content, as well as no impact on wheat yields compared to using synthetic N fertilizer.

MATERIALS AND METHODS

Crop Management

Experimental plots were established at the Oklahoma State University, North Central Research and Extension Center in Lahoma, OK on a Grant silt loam (fine-silty, mixed, superactive, thermic Udic Argiustolls) on a one to three percent slope. The treatment structure was arranged in a randomized complete block design with three replications. Cover crop mixtures contained the species presented in table 1 and were designed to contain either all legumes, all grasses, or a combination of legume and grass (Table 2). Cover crops were planted 5 July and 3 July in 2013 and 2014, respectively.

The cover crops were terminated with glyphosate (N-[phosphonomethyl]-glycine), and 2-4-D (2, 4-dichlorophenoxyacetic acid) in accordance with label specifications on 15 August 2013 and 11 August 2014. Paraquat (1, 1'-dimethyl-4, 4'-bipyridinium dichloride) was used in an effort to terminate plants that did not die as a result of standard applications of glyphosate and 2-4-D.

All plots were fertilized with 36 kg N ha⁻¹ and 51 kg P₂O₅ ha⁻¹ applied as a combination of ammonium nitrate (AN, 34-0-0) and di-ammonium phosphate (DAP, 18-46-0) on 26 September 2013, prior to wheat planting. Wheat was planted with a John Deere 1590 no-till drill (Deere and Company, Moline, IL) on 18 October and 22 October in 2013 and 2014, respectively. Wheat was harvested using a Massey Ferguson combine with a two meter wide cutting table. In 2014, 12 kg N ha⁻¹ as DAP was applied in the seed furrow at wheat planting. In 2013, the wheat was top-dressed with urea ammonium nitrate (UAN,

28-0-0) on 14 March. Cover crop treatments received 36 kg N ha⁻¹, and fallow treatments were top-dressed according to treatment rates (Table 2) at GS 31 (Zadoks, et al., 1974).

Soil Sampling

Prior to establishment of the cover crop treatments (July 2013), soil samples were collected to a depth of 110 cm with a tractor mounted hydraulic probe (Giddings Machine Company, Windsor, CO). Soil cores were divided into depth increments from 0-10, 10-20, 20-40, 40-80, and 80-110 cm. Soil cores were again collected prior to planting of the wheat crop in Sept 2013, in Feb 2014, and following wheat harvest in June 2014. Each sample was analyzed for soil moisture content and bulk density. Surface samples (0-40 cm) were collected at wheat planting in 2014 and analyzed for soil nitrate (NO₃). Soil nitrate was analyzed via flow injection analysis (QuickChem FIA+, Lachat Instruments, Milwaukee, WI) after extraction with 1 mol⁻¹ L KCl (1:5 soil/KCl).

Cover Crop Sampling

Prior to termination, cover crops were sampled for biomass yield and biomass N content by randomly selecting a 1 m² area and clipping the biomass within the area to the soil surface and drying the biomass collected to determine yield on a dry weight basis.

Biomass nitrogen content was determined using a TrueSpec CN analyzer (LECO, Inc. St. Joseph, MI). A linear mixed model was used for statistical analyses of wheat grain yield, cover crop biomass yield, cover crop N content, and soil moisture where soil depth was treated as a repeated measure. and was performed using the Mixed procedure in SAS v. 9.4 (SAS Institute, 2008); means separation were performed using Fisher's Protected

LSD. Soil moisture depths were grouped into two layers, surface (0-40 cm) and subsoil (40-110 cm) and compared using contrast analysis.

RESULTS AND DISCUSSION

Wheat yield

Wheat yields for the 2013-2014 crop year were not significantly different for any treatment ($\alpha = 0.05$, $p = 0.5722$). Mean wheat yield ranged from 1152 to 1874 kg ha⁻¹ (Table 4). This lack of difference in the wheat yields is in agreement with Wortman, et al. (2012) which also found there to be no difference in cash crop yield following cover crop mixtures. Tonitto, et al. (2006) also found, in a meta-analysis of legume and non-legume cover crops, that many studies reported no change in cash crop yield following cover crops when fertilized at recommended fertilization levels.

Cover Crop Biomass

No interaction was found between year and treatment for either cover crop biomass yield or cover crop biomass N content, therefore biomass data from 2013 and 2014 were combined. There were no significant differences in cover crop biomass yield or cover crop biomass N content (Table 5). The lack of differences was unexpected as 2013 received 280 mm of rainfall during the cover crop growing season versus 140 mm of rainfall in 2014. One possible explanation for the lack of detectable differences could be the large coefficient of variation for the treatments, some treatments were approximately 50%. This variation likely resulted from variability in soil characteristics across the study which influenced the biomass produced. *Soil NO₃*

Significant differences ($\alpha=0.05$, $p=0.0027$) were found between treatments for soil NO_3 at wheat planting in 2014 (Table 6). The highest soil NO_3 concentrations were found in the UAN 72 and UAN 136 treatments. The remaining UAN treatments were not significantly different from the UAN 72 and UAN 136. Cover crop mixtures containing only legume species (Treatments 1, 3, and 5) had soil NO_3 concentrations that were not significantly different from the UAN treatments. However, cover crop mixtures containing grasses had significantly lower soil NO_3 concentrations from the UAN 72 and UAN 136 treatments. Kuo and Sainju (1998) found that when using mixtures containing hairy vetch, rye, and/or ryegrass, N immobilization was intensified when hairy vetch composed less than 40% of the mixture. The significantly lower soil NO_3 concentrations in the cover crop mixtures containing grasses could be a result of increased N immobilization by the grass species.

Soil Bulk Density and Moisture

No interaction was found between treatments and sample date for bulk density. There were no significant differences found between treatments when the data from all sample dates were combined.

Analysis of variance found a significant treatment by sample date interaction for soil moisture. However, for each soil sampling date there was no significant effect of treatment. Since there was no treatment effect, treatments were categorized by treatment type as either 'cover' or 'fallow' in order to run a contrast analysis on soil moisture content at the different sampling times for the surface (0-40 cm) and the subsoil (40-110 cm) (Figures 1-4). Wortman, et al. (2012) also reported surface (0-8 cm) soil moisture as

being unaffected by cover crop or lack of cover crop at termination; however, that is the only depth reported. Prior to wheat planting (Sept 2013) there was no significant difference between the cover crop and fallow treatments in the surface (0-40 cm) soil. However, in the subsoil (40-110 cm) the cover crop treatments were significantly drier than the fallow treatments ($\alpha=0.05$, $p=0.0016$) (Figure 2). This depletion of soil moisture seems to reinforce the concern producers state as a hindrance to implementing cover crops. However, by February, the soil moisture differences between the cover and fallow treatments were reduced with the cover crop treatments having no significant differences from the fallow treatments. Post-wheat harvest, the cover treatments are again not significantly different from the fallow treatments. Given that the yields among the treatment were not significantly different, this one year of data may indicate that the soil moisture reduction from the cover crop treatments at planting of the wheat may not have a negative impact on final yields.

CONCLUSIONS

Utilizing cover crop mixtures did not affect wheat yields compared to fallow treatments for the 2013-2014 crop year. Species composition did not significantly affect biomass yields of the cover crop treatments and despite differences in the amount of rainfall received during the cover crop growing seasons in 2013 and 2014 biomass yields were not significantly different in 2013 and 2014. The cover crop biomass N was not significantly different between cover crop mixtures, regardless of species composition and was generally proportional to biomass yield. At wheat planting in 2014, there were differences in soil NO₃ content among the treatments. The cover crop mixtures containing only legumes were had soil NO₃ concentrations as high as the UAN 72 and

UAN 136 treatments. The results of this study show limited potential for the use of cover crop mixtures in the southern Great Plains in rotation with wheat due to the lack of improvements in wheat yield and nitrogen availability of the system. However, the data from this study indicate that, though summer cover crop mixtures can deplete subsoil moisture, it does not mean that yields of the following cash crop will be reduced as is speculated by producers. Furthermore, the lack of improved N availability for the wheat following legume cover crops may be the result of drought conditions during the wheat production phase, which would have limited N mineralization. This highlights the challenge of managing cover crops in the southern plains as well as the need for long-term efforts to evaluate the impact of their inclusion into a continuous wheat production system.

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Table 1. Cover crop species used for mixtures with common and scientific names.

Common Name	Scientific Name
Buckwheat	<i>Fagopyrum esculentum</i>
Radish	<i>Raphanus sativus</i>
Sunflower	<i>Helianthus annuus</i>
Sorghum-sudan BMR	<i>Sorghum bicolor</i> x <i>S. bicolor</i> var. sudanese
Corn (sterile)	<i>Zea mays</i>
Pearl Millet	<i>Pennisetum glaucum</i>
Mung bean	<i>Vigna radiata</i> (L.) Wilczek
Laredo Soybean	<i>Glycine max</i> L.
German Millet	<i>Setaria italica</i> L.
Sunn Hemp	<i>Crotalaria juncea</i> (L) Tropic Sunn
Cowpea	<i>Vigna unguiculata</i>

Table 2. Treatment numbers, cover crop species composition, and N fertilization rates for 2013 and 2014.

Treatment	Species in Mixture
1	Cowpea, Sunn Hemp
2	Cowpea, Sunn Hemp, Buckwheat, G. Millet, Laredo Soybean
3	Cowpea
4	Cowpea, Mungbean, Laredo Soybean, Sorghum-Sudan, P. Millet
5	Cowpea, Mungbean, Sunn Hemp, Laredo Soybean
6	Cowpea, Sunn Hemp, Radish, G. Millet
7	P. Millet, Sorghum-Sudan, G. Millet
8	Cowpea, Sunn Hemp, Sterile Corn, Sorghum-Sudan, Sunflower
9	UAN 36 kg ha ⁻¹ (36/0) [†]
10	UAN 72 kg ha ⁻¹ (36/36)
11	UAN 103 kg ha ⁻¹ (36/67)
12	UAN 136 kg ha ⁻¹ (36/100)

[†] Values in parentheses are the pre-plant UAN rate followed by the top-dress UAN rate.

Table 3. Seeding rate (kg ha⁻¹) of each species in cover crop mixtures.

Species Mixture	Seeding Rate										
	Buckwheat	Radish	Sunflower	Sorghum- sudan	Corn	P. Millet kg ha ⁻¹	Mung bean	L. Soybean	G. Millet	Sunn Hemp	Cowpea
1										22.4	22.4
2	13.4							5.6	4.5	4.5	4.5
3											22.4
4				6.1		6.1	7.2	7.2			7.2
5							11.6	5.8		4.6	11.6
6		2.3							11.2	6.7	13.4
7				11.2		11.2			11.2		
8			5.6	5.6	5.6					5.6	11.3

Table 4. Wheat yields (kg ha⁻¹) from each treatment, 2013-2014 crop year.

Treatment	Species/N Rate	Wheat Yield (kg ha ⁻¹)
1	Cowpea, Sunn Hemp	1454
2	Cowpea, Sunn Hemp, Buckwheat, G. Millet, Laredo Soybean	1874
3	Cowpea	1425
4	Cowpea, Mungbean, Laredo Soybean, Sorghum-Sudan, P. Millet	1229
5	Cowpea, Mungbean, Sunn Hemp, Laredo Soybean	1533
6	Cowpea, Sunn Hemp, Radish, G. Millet	1555
7	P. Millet, Sorghum-Sudan, G. Millet	1613
8	Cowpea, Sunn Hemp, Sterile Corn, Sorghum-Sudan, Sunflower	1282
9	UAN 36	1705
10	UAN 72	1491
11	UAN 103	1152
12	UAN 136	1443

Table 5. Cover crop biomass yield (kg ha^{-1}) and biomass N across years.

Treatment	Species/N Rate	Biomass Yield (kg ha^{-1})	Biomass N (kg ha^{-1})
1	Cowpea, Sunn Hemp	4967	110
2	Cowpea, Sunn Hemp, Buckwheat, G. Millet, Laredo Soybean	5889	100
3	Cowpea	3837	70
4	Cowpea, Mungbean, Laredo Soybean, Sorghum-Sudan, P. Millet	3751	88
5	Cowpea, Mungbean, Sunn Hemp, Laredo Soybean	2818	52
6	Cowpea, Sunn Hemp, Radish, G. Millet	2630	38
7	P. Millet, Sorghum-Sudan, G. Millet	4500	67
8	Cowpea, Sunn Hemp, Sterile Corn, Sorghum-Sudan, Sunflower	4605	74

Table 6. Soil nitrate concentration ($\text{mg NO}_3 \text{ kg}^{-1}$) at wheat planting (22 October 2014).

Treatment	Species/ N Rate	Soil Nitrate $\text{mg NO}_3 \text{ kg}^{-1}$
1	Cowpea, Sunn Hemp	12.6 ^{ab†}
2	Cowpea, Sunn Hemp, Buckwheat, G. Millet, Laredo Soybean	8.3 ^b
3	Cowpea	13.4 ^{ab}
4	Cowpea, Mungbean, Laredo Soybean, Sorghum-Sudan, P. Millet	6.3 ^b
5	Cowpea, Mungbean, Sunn Hemp, Laredo Soybean	12.2 ^{ab}
6	Cowpea, Sunn Hemp, Radish, G. Millet	8.8 ^b
7	P. Millet, Sorghum-Sudan, G. Millet	6.9 ^b
8	Cowpea, Sunn Hemp, Sterile Corn, Sorghum-Sudan, Sunflower	8.2 ^b
9	UAN 36	12.1 ^{ab}
10	UAN 72	20.5 ^a
11	UAN 103	14.3 ^{ab}
12	UAN 136	20.0 ^a

†Means followed by the same letter are not significantly different according to LSD ($\alpha=0.05$).

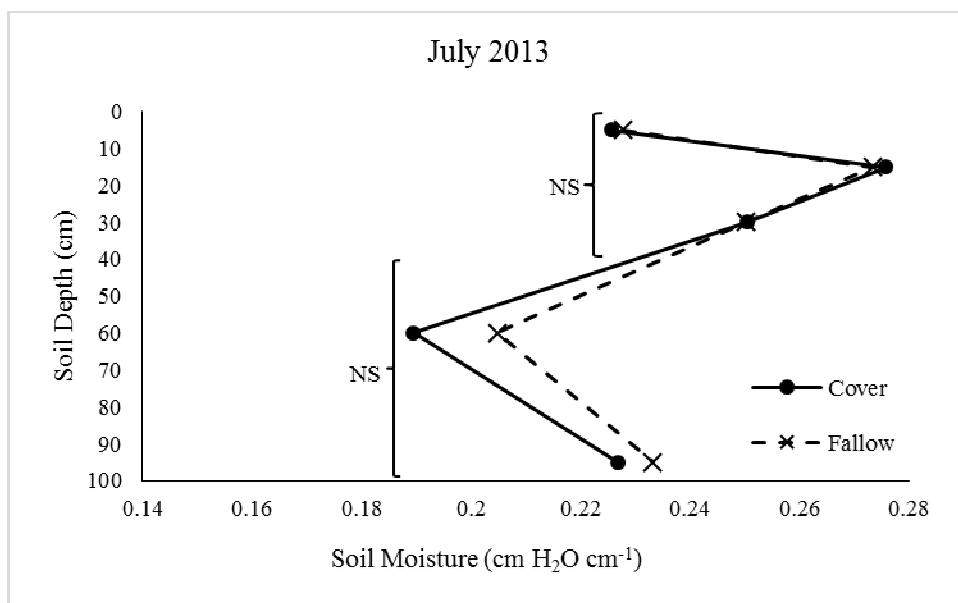


Figure 1. Average soil moisture content (cm H₂O cm⁻¹) of cover treatments and fallow treatments at cover crop planting July 2013. Brackets indicate soil layers where the top bracket is the surface soil (0-40 cm) and the bottom bracket is the subsoil layer (40-110 cm). No significant difference at the p=0.05 probability level is indicated by NS.

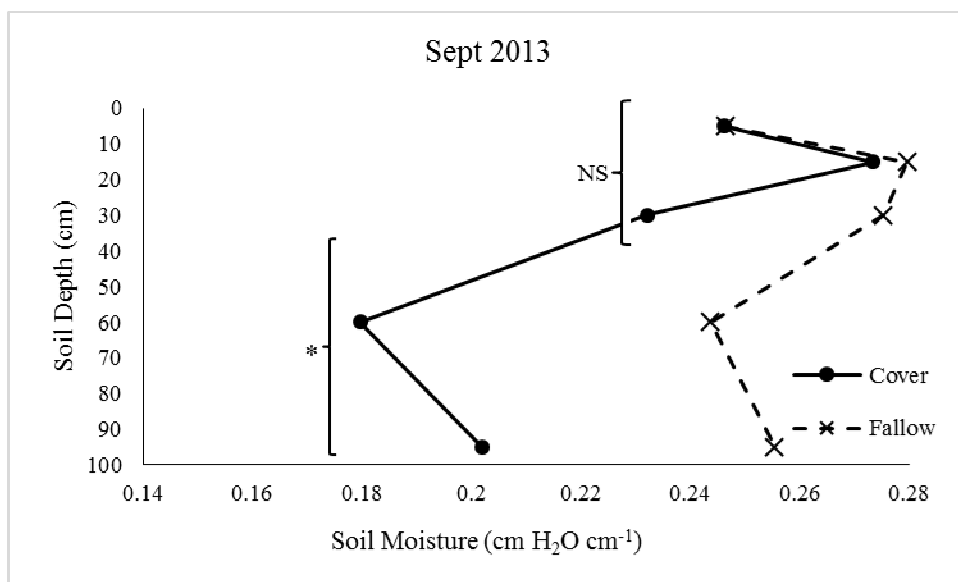


Figure 2. Average soil moisture content (cm H₂O cm⁻¹) of cover treatments and fallow treatments at wheat planting September 2013. Brackets indicate soil layers where the top bracket is the surface soil (0-40 cm) and the bottom bracket is the subsoil layer (40-110 cm). No significant difference at the p=0.05 probability level is indicated by NS. An asterisk (*) indicates significance at p=0.05.

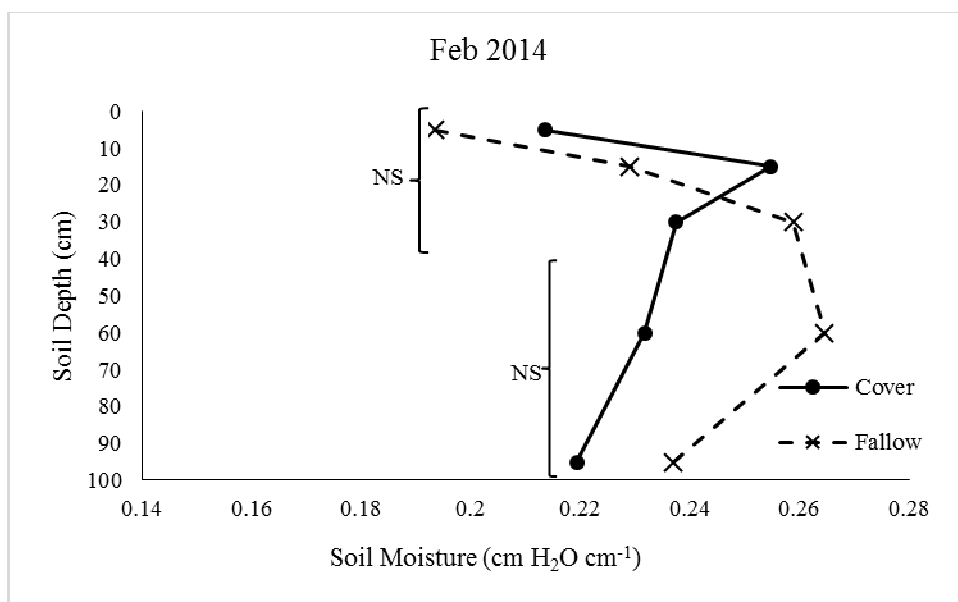


Figure 3. Average soil moisture content (cm H₂O cm⁻¹) of cover treatments and fallow treatments in February 2014. Brackets indicate soil layers where the top bracket is the surface soil (0-40 cm) and the bottom bracket is the subsoil layer (40-110 cm). No significant difference at the p=0.05 probability level is indicated by NS.

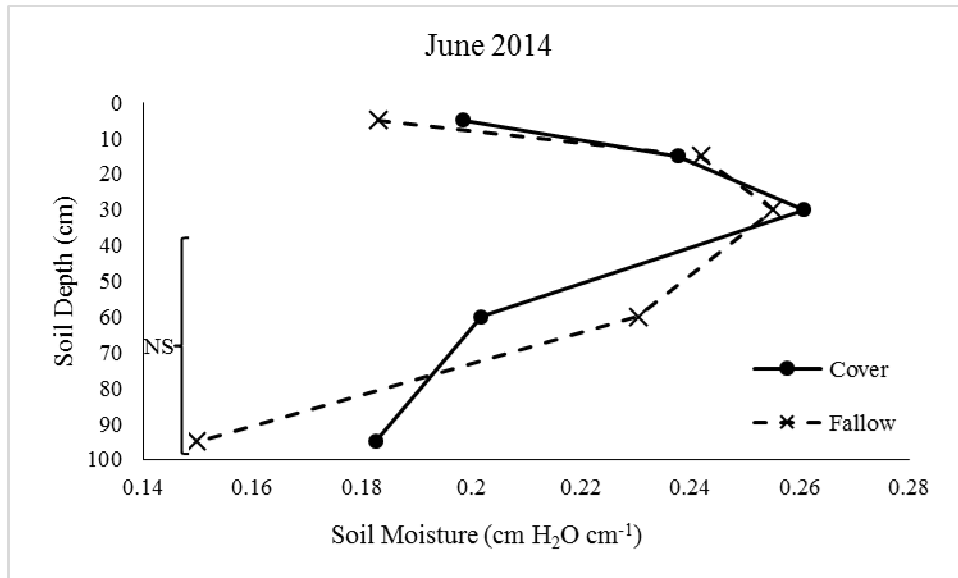


Figure 4. Average soil moisture content (cm H₂O cm⁻¹) of cover treatments and fallow treatments after wheat harvest in June 2014. Brackets indicate soil layers where the top bracket is the surface soil (0-40 cm) and the bottom bracket is the subsoil layer (40-110 cm). No significant difference at the p=0.05 probability level is indicated by NS.

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